Reitstötter, Kinzebach & Partner (GbR)
Patentanwälte

Reitstötter, Kinzebach & Partner
Postfach 86 06 49, D-81633 München

Dr. Werner Kinzebach
Dr. Peter Riedl
Dr. Georg Schweiger
Dr. J. Uwe Müller
Dr. Wolfgang Thalhammer
Dr. Michael Pohl
Dr. Thomas Wolter
Andreas Rabe
Dr. Jens Wortmann
Prof. Dr. Dr. Reitstötter (1982)

Zugelassene Vertreter beim
Europäischen Patentamt
European Patent Attorneys
Telefon: +49(0)89 99 83 97 - 0
Telefax: +49(0)89 98 73 04
Sternwartstr. 4, D-81679 München
email: office@kinzebach.de

An das
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80298 München

München, 14.06.2005

Unsere Akte.: M/45356-OPPO

Re.: **Einspruch ./. EP 0 656 786 (Anmeldung Nr. 93909679.8)**
Patentinhaber: Novogen Research Pty Ltd
Einsprechende: Apomedica Pharmazeutische Produkte GmbH et al.

Im Namen und Auftrag der

1)

Apomedica Pharmazeutische Produkte GmbH
Rosseggerkai 3, A-8011 Graz
Österreich

2)

Salus Haus Dr. med. Otto Greither Nachf. GmbH & Co. KG
Bahnhofstr. 24, D-83052 Bruckmühl
Deutschland

3)

Astrid Twardy GmbH
Feringastr. 4, D-85774 Unterföhring
Deutschland

Zur Kasse
A. 610,-

4)

Fa. Anton Hübner GmbH & Co. KG
Schloßstr. 11-17, D-79238 Ehrenkirchen
Deutschland

5)

Bad Heilbrunner Naturheilmittel GmbH & Co.
Am Krebsenbach 5-7; D-83670 Bad Heilbrunn
Deutschland

wird

Einspruch (gemeinsamer Einspruch)

gegen das europäische Patent EP 0 656 786 der Novogen Research Pty Ltd. mit dem Titel "Verwendung von Isoflavon Phyto-Östrogen Extrakten von Soja oder Klee" eingelegt.

Die unter 1) genannte Einsprechende ist gemeinsamer Vertreter der Einsprechenden. Der gemeinsame Vertreter hat den unterzeichnenden Patentvertreter bestellt, für die Einsprechenden vor dem Europäischen Patentamt zu handeln.

Die Einspruchsgebühr in Höhe von EUR 610,00 wird mit dem beiliegenden Abbuchungsauftrag (EPA Form 1010) entrichtet.

Es wird beantragt, das angegriffene Patent in vollem Umfang zu widerrufen. Mündliche Verhandlung wird beantragt, falls die Einspruchsabteilung beabsichtigt, das Patent unverändert oder in eingeschränkter Fassung aufrecht zu erhalten.

Wie nachstehend ausgeführt, geht der Gegenstand des angegriffenen Patent über die Anmeldung in der eingereichten Fassung hinaus (Art. 100 c EPÜ), ist nicht neu bzw. beruht – soweit neu – nicht auf einer erfinderischen Tätigkeit (Art. 100 a EPÜ).

Tatsachen und Begründung

I. Entgegenhaltungen

D1 Song et al., J Agric Food Chem 47:1607-1610 (1999) (Zusammenfassung)

D2 WO 94/23716

D3 EP 0 135 172, veröffentlicht am 27. März 1985

D4 JP62106016 (Zusammenfassung)

D5 Cheng et al., Science 118:164-165 (1953)

D6 Schrage, Therapie des klimakterischen Syndroms, VHC Verlagsgesellschaft, Weinheim 1985, Seite 11-12 und 99-101

D7 Lindner, Environ Qual Saf Suppl. 5:151-158 (1976)

D8 Duke, Medicinal plants of China. In: Medicinal plants of the world; No.4, Reference Publications, Inc., Algonac 1985, Seite 343-344

D9 Beckham, Australian Wellbeing 29:74-76 (1988)

II. Einspruchsgründe

1. Vorbemerkung zur Auslegung des Begriffs "Isoflavon-Phytoöstrogen-Extrakt"

Unter dem Begriff "Extrakt" ist nach fachmännischer Auffassung ein Auszug zu verstehen, der als das Ergebnis einer Einwirkung eines Extraktionsmittels oder einer mechanischen Kraft auf das Ausgangsmaterial anzusehen ist. Der Begriff "Extrakt" ist im angegriffenen Patent nicht weiter spezifiziert, beispielsweise durch Angabe der Extraktionsbedingungen oder eines Extraktionsmittels, die Angabe von Wertsubstanzen oder der Beschaffenheit. Der Begriff "Isoflavon-Phytoöstrogen-Extrakt" ist somit breit auszulegen als ein Material, das (prinzipiell) aus Soja oder Klee extrahierbar ist und in irgendeiner Form Phytoöstrogen-Isoflavone enthält. Die Spanne der unter diesen Begriff fallenden Substanzen bzw. Substanzgemische reicht somit von Extrakten mit einer Vielzahl von nativen Pflanzenbestandteilen, bei denen die Isoflavone (in der Literatur auch als "Isoflavonoide" bezeichnet) nur in geringer Konzentration vorhanden sind, bis hin zu hochangereicherten Extrakten sowie (als Phytoöstrogene wirksamen) Isoflavonen als Reinsubstanzen, sofern diese in Klee oder Soja vorkommen. Demzufolge fällt unter den Begriff eines solchen Isoflavon-Phytoöstrogen-Extrakts beispielsweise auch reines Genistein oder

Daidzein.

2. Unzulässige Erweiterung (Art. 100c EPÜ)

Anspruch 1 in der ursprünglich eingereichten Fasssung lautet wie folgt:

> *"A health supplement comprising a health supplementary amount of a phyto-oestrogen selected from genistein, daidzein, biochanin A, and/or formononetin."*

Daraus geht zwingend hervor, dass ausschließlich Zusammensetzungen in Betracht gezogen wurden, die mindestens eine der Verbindungen Genistein, Daidzein, Biochanin A oder Formononetin enthalten.

Anspruch 1 des erteilten Patents lautet dagegen wie folgt:

> *"The use of an isoflavone phyto-oestrogen extract of soy or clover, for the manufacture of a medicament for administration in unit dosage form for the treatment of pre-menstrual syndrome, sypmtoms associated with menopause, or prostate cancer."*

Demnach kann der Extrakt ein beliebiges Isoflavon-Phytoöstrogen enthalten, sofern das Isoflavon-Phytoöstrogen aus Soja oder Klee extrahierbar ist. Der Anspruch umfasst somit auch Ausführungsformen, die lediglich ein oder mehrere von Genistein, Daidzein, Biochanin A bzw. Formononetin verschiedene Isoflavon-Phytoöstrogene enthalten.

Wie nachfolgend gezeigt wird, sind die in Soja vorkommenden Isoflavon-Phytoöstrogene nicht auf die Verbindungen Genistein, Daidzein, Biochanin A oder Formononetin beschränkt.

D1 identifiziert Glycitein eindeutig als Isoflavon mit Östrogenwirkung und beschreibt dessen Aufreinigung aus Soja (Zusammenfassung). Es ist somit möglich, einen Isoflavon-Phytoöstrogen-Extrakt aus Soja herzustellen, der zwar weder Genistein, Biochanin A, Daidzein noch Formononetin enthält, dafür aber ein anderes Isoflavon-Phytoöstrogen, nämlich Glycitein. Die Verwendung dieses Extrakts fiele unter den erteilten Anspruch 1. Da Extrakte, in denen keine der Verbindungen Genistein,

Daidzein, Biochanin A und Formononetin vorkommt, in der ursprünglich eingereichten Fassung nicht offenbart waren, liegt eine unzulässige Erweiterung gemäß Art. 123(2) EPÜ vor.

3. Mangelnde Neuheit und erfinderische Tätigkeit von Anspruch 1

3.1. Gegenstand von Anspruch 1

Dargestellt in Form einer Merkmalsanalyse betrifft Anspruch 1 des angegriffenen Patents die

(1) Verwendung ... für die Herstellung eines Medikaments zur Verabreichung in Dosierungseinheitsform für die Behandlung
(2.1) des prämenstruellen Syndroms,
(2.2) von Symptomen, die mit der Menopause verbunden sind, oder
(2.3) von Prostatakrebs
(3) eines Isoflavon-Phytoöstrogen-Extraktes
(4.1) von Soja oder
(4.2) Klee.

3.2. Neuheit (Art. 54 EPÜ) von Anspruch 1

3.2.1. Der Gegenstand von Anspruch 1 ist nicht neu gegenüber D2

Dem Anspruch 1 kommt die beanspruchte Priorität vom 19. Mai 1992 nicht zu.

Der einzige Hinweis auf die Möglichkeit einer Extraktion in der Prioritätsunterlage findet sich auf Seite 8, Zeile 19 bis 24,

> *"Similarly, these materials may be used as a source of coumestans and isoflavones for further chemical or physical extraction or purification of those compounds leading to the further enrichment or eventual purification of these compounds for the purposes listed above."*

Die in Bezug genommenen Materialien ("these materials") sind

"... soya hulls only [...] or soya hypocotyls only [...] or a mixture of soya hulls and soya hypocotyls ..."

Von einer Verwendung der ganzen Sojabohne ist sogar ausdrücklich abgeraten (Seite 9, Zeile 3).

Die Prioritätsunterlage zieht also als Ausgangsmaterial für eine Extraktion lediglich Sojahülsen und/oder –kotyledonen in Betracht. Ein Extrakt aus **beliebigen** Teilen der Sojapflanze ist in der Prioritätsunterlage nicht offenbart.

Anspruch 1 in der erteilten Fassung differenziert nicht zwischen verschiedenen Teilen der Sojapflanze. Hinsichtlich des allgemeinen Begriffs "Isoflavon-Phytoöstrogen-Extrakt von **Soja**" ist die Priorität nicht wirksam beansprucht.

In der Prioritätsunterlage ist als alternative Isoflavonquelle nur **Erdklee** vorgesehen ("subterranean clover"; s. Seite 9, 3. Absatz). Der demgegenüber verallgemeinerte Begriff "Isoflavon-Phytoöstrogen-Extrakt von **Klee**" findet keine Grundlage.

Zusammenfassend ist festzustellen, dass dem Anspruch 1 in der erteilten Fassung nur der internationale Anmeldetag (19. Mai 1993) als Zeitrang zukommt.

D2 ist Stand der Technik gemäß Art. 54(3) EPÜ. Die Handlungen zur Einleitung der europäischen regionalen Phase wurden vorgenommen. D2 nimmt als Prioritätsdatum den 16. April 1993 in Anspruch. Der Offenbarung von D2 kommt vollumfänglich die beanspruchte Priorität zu.

D2 offenbart in Anspruch 1 die Verwendung von Isoflavonoiden für die Herstellung eines Medikaments zur Behandlung eines medizinischen Zustandes bei einer Frau (entsprechend Merkmal **(1)**), der durch verringerte oder veränderte Konzentrationen endogenen Östrogens hervorgerufen wird, wie z.B. vor der Menopause und dem prämenstruellen Syndrom (entsprechend den Merkmalen **(2.1)** und **(2.2)**). Dem Merkmal **(4.1)** entsprechend geht aus der Beschreibung von D2 weiterhin hervor, dass Soja eine geeignete Quelle für Isoflavonoide ist (Seite 1, Zeile 25, Zeile 32). Der Absatz auf Seite 2 (Zeile 12 bis 18) beschreibt einen mit Isoflavonoiden angereicherten Extrakt (Merkmal **(3)**) aus Soja (Merkmal **(4)**) in einem verzehrbaren Nahrungsmittelträger. Weitere Dosierungseinheitsformen ergeben sich aus dem letzten Absatz auf Seite 3.

Zusammenfassend nimmt D2 die Verwendung von Isoflavon-Extrakten aus Soja für die Herstellung von Medikamenten für die Behandlung von Menopause-Symptomen und prämenstruellem Syndrom vorweg.
Anspruch 1 des angegriffenen Patents ist somit durch D2 neuheitsschädlich getroffen.

3.2.2. Der Gegenstand von Anspruch 1 ist nicht neu gegenüber D3

D3 offenbart in Beispiel 2 Kapseln, d.h. Dosierungseinheitsformen, die 2,4'-Dihydroxyisoflavon, d.h. Daidzein, enthalten. Diese Dosierungseiheitsformen finden Anwendung zur Behandlung von Osteoporose (vgl. Anspruch 1 von D3). D3 ist weiter zu entnehmen (Seite 1, Zeile 14-15):

> *"Osteoporosis is a disease condition or illness which occurs frequently in postmenopausal females..."*

Es steht somit außer Frage, dass die **Verwendung von Daidzein** für die Herstellung eines Medikaments zur **Behandlung eines mit der Menopause assoziierten Symptoms** bereits bekannt war.

D3 gibt nicht an, dass Daidzein aus Soja oder Klee extrahiert ist. Daidzein ist eine chemisch genau umschriebene Verbindung. Der Anspruch erlangt durch die Angabe einer Quelle für das Daidzein keine Neuheit. Da Daidzein prinzipiell aus Soja oder Klee extrahiert werden kann, ist nach dem eingangs Gesagten reines Daidzein als "Isoflavon-Phytoöstrogen-Extrakt" aus Soja oder Klee anzusehen.

3.2.3. Der Gegenstand von Anspruch 1 ist nicht neu gegenüber D4

D4 offenbart die Verwendung von 5,7,4'-Trihydroxyisoflavon (Genistein) zur Linderung von Osteoporose. Wie oben festgestellt wurde und dem Fachmann ohne weiteres geläufig ist, ist Osteoporose ein Symptom, das mit der Menopause verbunden ist. Die Verbindung soll in einer Dosis von 200 - 1000 mg/Tag verabreicht werden.

D4 gibt nicht an, dass Genistein aus Soja oder Klee extrahiert ist. Da Genistein prinzipiell aus Soja oder Klee extrahiert werden kann, ist nach dem eingangs

Gesagten reines Genistein als "Isoflavon-Phytoöstrogen-Extrakt" aus Soja oder Klee anzusehen.

3.3. Mangelnde erfinderische Tätigkeit (Art. 56 EPÜ) von Anspruch 1

3.3.1 Mangelnde erfinderische Tätigkeiteit gegenüber D9

D9 ist ein Artikel mit der Überschrift "Herbal Help to Avoid Menopause Symptoms", der mit dem einleitenden Satz beginnt:

> *"The aim of this article is to provide information on non-harmful ways of overcoming the problems of menopause."*

In dem Absatz "What happens when the ovaries stop functioning?" (Seite 74) beschreibt die Autorin als wesentlichen Faktor für Menopausebeschwerden die erniedrigte Östrogenproduktion des Körpers sowie die Östrogen-Ersatztherapie als bei vielen Frauen angewandte Art der Medikation. Eine Überleitung zur Phytotherapie erfolgt auf Seite 74 (mittlere Spalte, letzter Absatz):

> *"Since the 1920s over 50 different species of plant have been found to contain oestrogenic substances."*

Schließlich wird auf Seite 74 (rechte Spalte) ist unter der Überschrift "Red Clover" die Verwendung von Rotklee zu diesem Zweck erwähnt. Als Dosierung wird ein Tee genannt. Tee ist ein wässriger Pflanzenextrakt. Rotklee enthält Isoflavon-Phytoöstrogene. Diese kommen in der Pflanze primär in der Glykosid-From vor, die wasserlöslich ist (vgl. das angegriffene Patent, Seite 2, Zeile 32, und Seite 8, Zeile 40).

Es ist fachübliches und naheliegendes Vorgehen, Pflanzenextrakte als Dosierungseinheitsformen bereitszustellen.

Anspruch 1 beruht somit nicht auf erfinderischer Tätigkeit.

3.3.2. Mangelnde erfinderische Tätigkeit gegenüber D4 und D5

Selbst wenn sich die Einspruchsabteilung wider Erwarten den unter Punkt 3.2.3 gemachten Ausführungen nicht anschließen sollte, so würde der Gegenstand von Anspruch 1 nicht auf einer erfinderischen Tätigkeit beruhen.

D4 kann als nächstliegender Stand der Technik angesehen werden, da diesem Dokument eine vergleichbare (subjektive) Aufgabe wie der Lehre des angegriffenen Patents zugrundeliegt. Darin ist die Verwendung von Genistein zur Behandlung Osteoporose gezeigt (eines konkreten Menopause-assoziierten Symptoms, wie unter Punkt 3.2.3 ausgeführt). Gegenüber D4 ist die dem angegriffenen Patent zugrundeliegende (objektive) Aufgabe als Bereitstellung einer alternativen Quelle für Genistein zu formulieren.

D5 beschreibt einen Weg, wie man durch ein Extraktionsverfahren aus Sojaschrot reines Genistein in Form rechteckiger Stäbchen mit einem Schmelzpunkt von 298°C herstellt (Seite 165, linke Spalte, 2. Absatz). Durch eine Kombination von D4 und D5 kommt der Fachmann also in naheliegender Weise zum Gegenstand von Anspruch 1.

3.3.3. Mangelnde erfinderische Tätigkeit gegenüber D6 und D7

Für die Behandlung des klimakterischen Syndroms, also auch von Menopause-assoziierten Symptomen, stellt D6 ein Standardlehrbuch für Gynäkologen dar. Ein vollständiges Kapitel dieses Lehrbuchs (Kapitel 11, Seite 99 bis 107) ist bereits der Behandlung klimakterischer Ausfallerscheinungen mit pflanzlichen Wirkstoffen gewidmet. Im letzten Absatz auf Seite 99 ist zu lesen:

> *"Klimakterisch bedingte Beschwerden mit pflanzlichen Wirkstoffen anzugehen, bedeutet einen Ausweg, der nicht nur bei Kontraindikationen der Hormontherapie Interesse verdient. Dabei kann man bei Verwendung pflanzlicher Präparate drei verschiedene Wirkungsstufen unterscheiden. [...] ..[S]chließlich können pflanzliche Präparate angewandt werden, die Substanzen enthalten,* ***die in ihrer Wirkung z.B. den chemisch definierten Östrogenen entsprechen."*** **[Hervorhebung hinzugefügt]**

Der Begriff "pflanzliches Präparat" impliziert dabei bereits die Gewinnung eines im

Sinne der beabsichtigten Hormon-Ersatztherapie wirksamen Agens aus Pflanzen, mithin einen "Extrakt". Weiterhin impliziert der Begriff "Präparat" auch das Bereitstellen in Dosierungseinheitsform.

Sieht man D6 als nächstliegenden Stand der Technik an, so würde die mit dem angegriffenen Patent zu lösende Aufgabe in der Suche nach weiteren Präparaten bestehen, die "in ihrer Wirkung z.B. den chemisch definierten Östrogenen entsprechen".

Diese Suche gestaltet sich nun für den Fachmann nicht schwierig. Bereits in den 70er Jahren war allgemein bekannt, dass Klee und Soja zu den Pflanzen mit Inhaltsstoffen mit östrogener Wirkung gehören. D7 beschreibt in der Zusammenfassung (Seite 151):

> *"More than 40 plant species have been shown to contain substances that are active in biological assays for estrogenic activity."*

Trifolium subterraneum, T. pratense und *Soya hispida* sind wörtlich im 2. Absatz auf Seite 151 genannt, die wesentlichen Inhaltsstoffe von Klee mit phyto-östrogener Wirkung, nämlich die Isoflavone Genistein, Biochanin A, Daidzein sowie auch Formononetin, im darauffolgenden Absatz.

Der Gegenstand von Anspruch 1 ergibt sich durch eine Kombination von D6 und D7 für den Fachmann somit in naheliegender Weise.

3.3.4. Mangelnde erfinderische Tätigkeit gegenüber D6 und D8

Anspruch 1 ist in breiter Form auf die Behandlung von prämenstruellem Syndrom und Menopause-assoziierten Symptomen, d.h. Merkmale **(2.1)** und **(2.2)**, gerichtet, ohne dass im angegriffenen Patent eine genaue Beschreibung der zugehörigen Krankheitsbilder gegeben wird. Als gynäkologisches Lehrbuch auf dem Gebiet der klimakterischen Beschwerden fasst D6 eine Vielzahl typischer Beschwerden und Veränderungen zusammen (Seite 11 bis 12), die nachfolgend auszugsweise wiedergegeben sind:

> *"Obstipation, [...] rheumatoide Beschwerden, [...] Reizbarkeit, Aggressivität, Stimmungslabilität, [...]"*

Klee ist eine traditionelle Heilpflanze in verschiedenen Ländern. D8 ist eine Zusammenfassung von Heilpflanzen, die in China verwendet werden, und beschreibt als Wirkungen von bzw. Indikationen für *T.pratense* u.a. (Seite 343):

"Aperient, [...] sedative; [...] constipation, [...] rheumatism"

Es ist ohne weiteres einsichtig, dass *T. pratense* beispielsweise in seiner Eigenschaft als Abführmittel ("aperient") für die Behandlung von Verstopfung sowie in seiner Eigenschaft als Beruhigungsmittel für die Behandlung von Reizbarkeit, Aggressivität und Stimmungslabilität geeignet ist. Zusammengefasst ist Rotklee somit für viele Menopause-assoziierte Beschwerden bereits als Therapeutikum bekannt. Eine Bereitstellung in Dosierungseiheitsform ist fachüblich.

Im Lichte von D6 und D8 beruht Anspruch 1 nicht auf erfinderischer Tätigkeit.

4. Abhängige Ansprüche 2 bis 11

Die abhängigen Ansprüche enthalten keine Merkmale, die geeignet sind, in Verbindung mit den Merkmalen des Anspruchs 1 Neuheit und erfinderische Tätigkeit herzustellen.

III. Schlußbemerkung

Es zeigt sich im Ergebnis, dass der eingangs gestellte Antrag auf vollständigen Widerruf begründet ist.

W. Thalhammer

D1

Erratum in:

- J Agric Food Chem 2002 Apr 10;50(8):2470.

Estrogenic activity of glycitein, a soy isoflavone.

Song TT, Hendrich S, Murphy PA.

Food Science and Human Nutrition, 2312 Food Sciences Building, Iowa State University, Ames 50011, USA.

Glycitein (4',7-dihydroxy-6-methoxyisoflavone) accounts for 5-10% of the total isoflavones in soy food products. The biological activity of this compound has not been reported to date, although numerous studies have been performed with the other soy isoflavones, daidzein and genistein. Glycitein was isolated from soy germ to 99% purity. Weaning female B6D2F1 mice were dosed with glycitein (3 mg/day), genistein (3 mg/day), and diethylstilbestrol (DES) (0.03 microg/day) in 5% Tween 80 by gavage for 4 days. A control group received an equal volume of 5% Tween 80 solution daily. The uterine weight increased 150% with glycitein ($p < 0.001$), 50% with genistein ($p < 0.$

001), and 60% with DES ($p < 0.001$) compared with the control group. DES, 17beta-estradiol, and three isoflavones (daidzein, genistein, and glycitein) were examined for their competitive binding abilities with 17beta-((3)H)estradiol to the estrogen receptor proteins of the B6D2F1 mouse uterine cytosol. The concentrations of each compound required to displace 50% of the ((3)H)estradiol at 5 nM in the competitive binding assay were 1.15 nM DES, 1.09 nM 17beta-estradiol, 0.22 microM genistein, 4.00 microM daidzein, and 3.94 microM glycitein. These data indicated that glycitein has weak estrogenic activity, comparable to that of the other soy isoflavones but much lower than that of DES and 17beta-estradiol.

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D2

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(71) Applicant: TUFTS UNIVERSITY SCHOOL OF MEDICINE [US/US]; 136 Harrison Avenue, Boston, MA 02111 (US).

(72) Inventors: GORBACH, Sherwood, L.; 429 Beacon Street, Chestnut Hill, MA 02115 (US). GOLDIN, Barry, R.; 38 Adella Avenue, West Newton, MA 02165 (US). ADLERCREUTZ, Herman; Department of Clinical Chemistry, University of Helsinki, Meilahti Hospital, FIN-00290 Helsinki (FI).

(74) Agent: CLARK, Paul, T.; Fish & Richardson, 225 Franklin Street, Boston, MA 02110-2804 (US).

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(54) Title: METHOD FOR TREATMENT OF MENOPAUSAL AND PREMENSTRUAL SYMPTOMS

(57) Abstract

A method is provided for preventing or treating symptoms of menopause, premenstrual syndrome, or a condition resulting from reduced levels of endogenous estrogen, by administering to the woman an effective amount of an isoflavonoid. The invention also features a therapeutic dietary product, containing isoflavonoids, for preventing or treating symptoms of conditions resulting from reduced or altered levels of endogenous estrogen.

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METHOD FOR TREATMENT OF MENOPAUSAL AND PREMENSTRUAL SYMPTOMS

Background of the Invention

The present invention relates to therapies for the prevention and treatment of menopausal and premenstrual symptoms.

It has long been recognized that the sharp reduction in endogenous estrogen levels which occurs prior to menopause causes a variety of unpleasant symptoms, e.g., hot flashes, nausea, nervousness, and malaise. Currently, the symptoms of menopause are treated by estrogen replacement therapy, which has recently been shown to increase the risk of certain types of cancer, such as endometrial cancer and breast cancer. Changes in levels of endogenous estrogen may also be responsible for "premenstrual syndrome", a condition occuring in younger women prior to menstruation. Premenstrual symptoms are treated with a variety of hormonal and nonhormonal therapies, which may cause side effects. Safer and more effective therapies for both conditions continue to be sought.

Summary of the Invention

The inventors have found that isoflavonoids, which are constituents of soy beans and other plants, effectively reduce the symptoms of conditions which are caused by reduced or altered levels of endogenous estrogen, e.g., menopause, and premenstrual syndrome. Without being bound by any theory, it is believed that the isoflavonoids bind to estrogen receptors, and thus exert an estrogenic response. These compounds, which are present naturally in soy-based and other plant-based foods, are safe and cause no significant side-effects. Isoflavonoids which may be administered according to the invention include genistein, daidzein, Biochanin A,

formononetin, O-desmethylangolensin, and equol; these may be administered alone or in combination.

Accordingly, in one aspect, the invention features a method of preventing or treating the symptoms of menopause, premenstrual syndrome, or a condition resulting from reduced levels of endogenous estrogen, by administering to the woman an effective amount of at least one isoflavonoid. The isoflavonoid may be administered in any suitable form, e.g., in the form of a plant extract rich in isoflavonoids or in the form of a purified or synthesized isoflavonoid.

In another aspect, the invention features a therapeutic dietary product for preventing or treating symptoms resulting from reduced or altered levels of endogenous estrogen. The dietary product preferably includes a soy extract containing enriched isoflavonoids, provided in a palatable food carrier, e.g., a confectionary bar, biscuit, cereal or beverage.

Other features and advantages of the invention will be apparent from the Description of the Preferred Embodiments thereof, and from the claims.

Description of the Preferred Embodiments

Isoflavonoids are naturally occurring substances, found primarily in soy beans. These compounds are also found in lower concentrations in many other plants. Isoflavonoids can thus be administered to a patient by placing the patient on a diet containing high levels of soy-based food products, e.g., tofu, miso, soybeans, aburage, atuage and koridofu, or other plant products rich in isoflavonoids.

These products may not be readily available in all geographic regions (most of these foods are served predominantly in Japan), and are not be palatable to many women, particularly those accustomed to Western-style food.

Accordingly, an isoflavonoid-containing fraction can be extracted from a soy or plant product. It is preferred that the isoflavonoids be extracted and concentrated from soy bean or soy powder. Isoflavonoids are also available commercially in substantially pure form. The concentrated isoflavonoid is preferably included in a food carrier to form a dietary product. Any type of palatable carrier may be used, but, as the isoflavonoid concentrate has a strong flavor, it is preferred that the carrier include suitable flavorings to impart a different, more palatable flavor. The dietary product may be any type of food product, e.g., a confectionary bar, biscuit, cereal or beverage.

It is preferred that the dietary product contain at least 30 mg/serving total isoflavonoids. The isoflavonoid concentrate included in the dietary product preferably includes a blend primarily comprised of genistein and daidzein. The concentrate typically also contains lower levels of other isoflavonoids. Most preferably, the dietary product contains from about 10 to 30 mg/serving, more preferably about 20 mg/serving of genistein, and from about 5 to 10 mg/serving, more preferably about 7 mg/serving of daidzein. Preferably, a dietary product containing the preferred dosage of isoflavonoids would be consumed at least once per day, preferably 1 to 2 times per day depending upon the severity of the woman's symptoms.

While it is preferred that the isoflavonoid be administered in the form of a dietary product, if desired the isoflavonoid could be administered, preferably in similar dosages, in medicament form, e.g., mixed with a pharmaceutically acceptable carrier to form a tablet, powder or syrup.

Example

The connection between diet and estrogen excretion was studied in Japanese women and men, and in a few children. The women's mean age was 50.4 (SD 18.0) years and they were all from a small village south of Kyoto and consumed a traditional Japanese low-fat diet. Isoflavonoid excretion in the urine was measured in a group of three men, three women, and three children living in Kyoto and consuming the traditional diet. We found a very high excretion of isoflavonoids in the urine of these subjects. The mean values were almost identical in the two groups and especially high excretion was found for genistein (maximum 15.5 umol per 24h in a man) and two other isoflavonoids, daidzein and equol (Table 1). All these compounds bind to estrogen receptors and have weak estrogenic activity. The excretion of the isoflavonoids in urine of the Japanese women was much higher than previously determined levels in American and Finnish women (Table 1). Excretion was high in children as in middle-aged and old people. These compounds were excreted in 100-fold to 1000-fold higher amounts than the levels of endogenous estrogens excreted by normal omnivorous women consuming a western or oriental diet (Table 1).

The excretion of the isoflavonoids in urine was associated with intake of soy products such as tofu, miso, aburage, atuage, koridofu, soybeans, and boiled beans.

It is known that Japanese women have a lower incidence of menopausal symptoms and premenstrual symptoms than the American and Finnish women.

Table 1

Urinary isoflavonoid or estrogen (nmol/day)	Japanese/ Oriental	American	Finnish
Genistein	3440(n=3)	. .	32.1(n=12)
Daidzein	2600(n=10)	216(n=21)	40.5(n=12)
Equol	2600(n=10)	62.8(n=21)	44.2(n=12)
Oestrone (postmenstrual)	4.48(n=9)	. .	4.48(n=10)
Oestradiol (postmenstrual)	0.76(n=9)	. .	0.94(n=10)
Oestriol (postmenstrual)	4.48(n=9)	. .	4.44(n=10)

CLAIMS

1. Use of an isoflavonoid in the preparation of a medicament for preventing or treating a medical condition in a woman caused by reduced or altered levels of endogenous estrogen.

2. The use of claim 1, wherein said isoflavonoid is selected from the group consisting of genistein, daidzein, Biochanin A, formononetin, O-desmethylangolensin and equol.

3. The use of claim 1 wherein said isoflavonoid is in a unit dosage of at least 30 mg.

4. The use of claim 1 wherein genistein and daidzein isoflavonoids are present in said medicament.

5. The use of claim 4 wherein said isoflavonoid comprises from about 10 to 30 mg genistein and from about 5 to 10 mg daidzein.

6. The use of claim 1 wherein said medicament is in the form of a dietary product.

7. The use of claim 6 wherein said dietary product contains at least 30 mg/serving of said isoflavonoid.

8. The use of claim 6 wherein said dietary product is a confectionery bar containing said isoflavonoid.

9. The use of claim 6 wherein said dietary product is a cereal containing said isoflavonoid.

10. The method of claim 6 wherein said dietary product is a biscuit containing said isoflavonoid.

11. The method of claim 6 wherein said dietary product is a beverage containing said isoflavonoid.

12. A dietary product for preventing or treating symptoms of menopause, premenstrual syndrome, or conditions resulting from reduced or altered levels of endogenous estrogen, comprising at least one isoflavonoid provided in a non-soy-based palatable food carrier.

13. The dietary product of claim 12 comprising genistein and daidzein isoflavonoids.

14. The dietary product of claim 12 wherein the food carrier is a confectionery bar.

15. The dietary product of claim 12 wherein the food carrier is a cereal.

16. The dietary product of claim 12 wherein the food carrier is a biscuit.

17. The dietary product of claim 12 wherein the food carrier is a beverage.

18. The dietary product of claim 12 wherein the food carrier contains an amount of the isoflavonoid which is effective in reducing the symptoms.

19. The dietary product of claim 18 comprising at least about 30 mg isoflavonoids per serving.

20. The dietary product of claim 13 wherein said dietary product comprises from about 10 to 30 mg/serving genistein and from about 5 to 10 mg/serving daidzein.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/04189

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : A61K 31/35-
US CL :514/456, 899

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/456, 899

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS AND CAS ONLINE: ISOFLAVIN?, PMS, ESTRO?, PREMENSTRUAL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US, A, 3,864,362 (FEUER ET AL.) 04 FEBRUARY 1975, COLUMN 1, LINE 33 - COLUMN 2, LINE 44.	1-20 ------ 1-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date		
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search 24 MAY 1994	Date of mailing of the international search report JUL 20 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer KIMBERLY JORDAN Telephone No. (703) 308-1235

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D3

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(71) Applicant: Takeda Chemical Industries, Ltd.
27, Doshomachi 2-chome Higashi-ku
Osaka-shi Osaka, 541(JP)

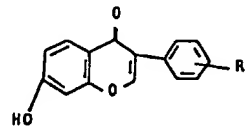
(72) Inventor: Yamazaki, Iwao
9-21, Hibarigaokayamate 1-chome
Takarazuka Hyogo 665(JP)

(72) Inventor: Sawa, Yoichi
19-11, Sengokuhigashi-machi
Kadoma Osaka 571(JP)

(74) Representative: von Kreisler, Alek, Dipl.-Chem. et al,
Deichmannhaus am Hauptbahnhof
D-5000 Köln 1(DE)

(54) **Method for treatment of osteoporosis.**

(57) A compound of the formula



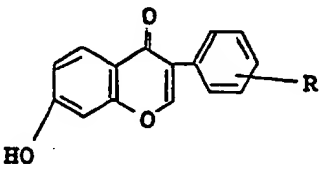
wherein R is a hydrogen atom or a hydroxy group is effective for prevention or treatment of osteoporosis.

EP 0 135 172 A2

Method for treatment of osteoporosis

This invention relates to a therapeutic means for treatment of osteoporosis.

More particularly, this invention relates to a medicament for prevention or treatment of osteoporosis, which contains a compound of the formula



wherein R is a hydrogen atom or a hydroxy group.

Osteoporosis is a disease condition or illness which occurs frequently in postmenopausal females, particularly those in their sixties, and wherein the quantitative loss of bones progresses beyond a certain limit to thereby present some symptoms or risk manifestations. Among its main clinical manifestations are kyphosis, low back pain, and fractures of femoral neck, lower end of the radius, ribs, upper end of the humerus, etc. While the causative factors are variegated, including endocrine disorder and nutritional disorder, apparently the most important cause is a descreased secretion of estrogen due to hypoovarianism in females during the postmenopausal period. Therefore, of all the therapeutic agents for osteoporosis, the theoretically most effective drugs are estrogen preparations.

However, the estrogens so far available are so strong in effect as to cause side effects such as genital bleeding, mastodynia, hepatic disorder, etc. and, for this reason, have not been used recently on as many occasions as in the past. There are other types of therapeutic agents such as calcitonin, vitamin D and calcium preparations, which however are disadvantageous in that they are either only indefinitely effective or ineffective when administered by the oral route.

The present inventors have found that the compound of the formula (I) exhibits a milder estrogen activity than the conventional estrogens in the oral regimen and does not cause side effects which are produced by these known drugs but cures osteoporosis by stimulating secretion of calcitonin from the thyroid.

The compounds of the formula (I) which are employed in accordance with the present invention are invariably crystalline compounds which are white to pale yellowish brown in color, and are freely soluble in dimethylformamide and chloroform, soluble in ethanol and acetone and practically insoluble in water. When R in the formula (I) is a hydroxy group, it may be present in any position of the phenyl ring.

These compounds can be produced, for example, by cyclizing a 2,4-dihydroxy-phenyl (with or without a hydroxy group on the benzene ring) benzyl ketone to a benzopyran compound, and some of these compounds are known to have capillary vessel stabilizing activity (French Pharmaceutical Patent No. 1065), therapeutic effective for vascular disorders, inflammatory and vitamin-P deficiency disorders (United States Patent No. 3,352,754) or anticonvulsant activity (Japanese Patent Publication No. 32074/1972), but it has not been known that any of the compounds is useful for the treatment of osteoporosis.

As will be apparent from Test Example 5 which appears hereinafter, all of the compounds of the formula (I) are

sparingly toxic. Thus, in the studies in which the compounds were administered orally or subcutaneously to mice or rats at the technically feasible highest doses (5,000 to 10,000 mg/kg), there occurred no death nor toxic symptoms attributable to the compounds.

On the other hand, Test Examples 1 and 2 presented hereinafter show that 7-hydroxy-isoflavone [hereinafter referred to briefly as compound (I)] and 7,4'-dihydroxy-isoflavone [briefly, compound (II)], which are representative species of the compound represented by the formula (I), have mild estrogenic activity which is suited for the treatment of osteoporosis.

Test Example 1

Estrogenic activity of 7-hydroxy-isoflavone in young oophorectomized rats

Sprague-Dawley rats, 33 days old and 11 days after oophorectomy for elimination of endogenous estrogenic activity, were used in groups of 6 to 7 animals. Compound (I) was suspended in a 1% aqueous solution of hydroxypropylcellulose and administered orally for 3 days, while as a representative example of the conventional estrogen drug, estrone was dissolved in sesame oil and administered subcutaneously for 3 days. On the fourth day, each animal was autopsied and its uterine wet weight was recorded. As shown in Table 1, compound (I) at the daily dose levels of 200 mg/kg and 400 mg/kg produced uterine weight increasing effect with a dose-response curve of moderate gradient. In contrast, estrone showed uterine weight increasing effect with a dose-response curve of steep gradient.

Table 1

Compound	Daily dose (mg/kg)	No. of animals	Uterine wet weight (mg ± S.D.)
Compound (I)	0 (control group)	7	35.0 ± 1.0
	6.25	7	32.8 ± 1.1
	12.5	7	33.4 ± 0.9
	25	7	35.1 ± 0.8
	50	7	35.3 ± 1.7
	100	7	35.9 ± 1.0
	200	7	57.9 ± 1.0*
	400	6	70.4 ± 6.7*
Estrone	0.0025	7	101.7 ± 4.6*
	0.005	7	159.8 ± 9.4*
	0.01	7	223.3 ± 12.5*

*: Significant as compared with control group ($P<0.01$)

Test Example 2

Estrogenic activity of 7,4'-dihydroxy-isoflavone in young oophorectomized rats

Sprague-Dawley rats, 33 days old and 11 days after oophorectomy for elimination of endogenous estrogenic activity, were used in groups of 7 animals. Compound (II) was suspended in a 1% aqueous solution of hydroxypropylcellulose and administered orally. As shown in Table 2, compound (II) at the dose level of 400 mg/kg showed mild uterine weight increasing activity.

Table 2

Daily dose of compound (II) (mg/kg)	No. of animals	Uterine wet weight (mg ± S.D.)
0 (control group)	7	31.1 ± 1.1
6.25	7	33.2 ± 0.8
25	7	32.8 ± 1.0
100	7	35.3 ± 1.3
400	7	62.3 ± 6.0*

*: Significant as compared with control group ($P<0.01$)

The following Test Examples 3 and 4 show that the compounds of this invention have bone resorption-inhibiting activity which is effective for the treatment of osteoporosis.

Test Example 3

Bone resorption inhibiting activity of 7-hydroxy-isoflavone and 7,4'-dihydroxy-isoflavone in rat fetal long bone culture.

Determination of bone resorption was performed by the method of Raisz [J. Clin. Invest. 44, 103-116 (1965)]. Thus, a Sprague-Dawley rat on the 19th day of pregnancy was subcutaneously injected with 50 μ Ci of ^{45}Ca (isotope of calcium, $CaCl_2$ solution), and was laparotomized on the following day. The embryos were aseptically taken out, the forelimbs (radius and ulna) were cut off from the trunk under a binocular dissecting microscope, and the connective tissue and cartilage were removed as much as possible to prepare bone samples. Each bone sample was preincubated at 37°C for 24 hours in 0.6 mℓ of the medium containing 2 mg/mℓ of bovine serum albumin in BGJ_b medium (Fitton-Jackson modification) [GIBCO Laboratories, Grand Island, NY 14072 U.S.A.]. Then, the sample was further incubated for 3 days in the same medium as above in which 10 μg/mℓ or 25 μg/mℓ of compound (I) or 10 μg/mℓ of compound (II) had been incorporated. Then, the radioactivity of ^{45}Ca in the medium and that of ^{45}Ca in the bone were measured and the percentage (%) of

^{45}Ca released from the bone into the medium was calculated by the following formula.

Percentage (%) of ^{45}Ca released from bone into medium

$$= \frac{\text{Count of } ^{45}Ca \text{ in medium}}{\text{Count of } ^{45}Ca \text{ in medium + Count of } ^{45}Ca \text{ in bone}} \times 100$$

As control, the bones of the embryos from the same litter were similarly incubated in the absence of compound (I) or (II) for 3 days. The mean ± standard deviation for the six bones per group are shown in Table 3. It is apparent that compounds (I) and (II) suppressed bone resorption.

Table 3

	Concentration of compound	^{45}Ca (%) released	
Control group	0	20.6 ± 3.8	19.9 ± 5.0
Test group 1	Compound (I) 10 µg/mℓ	16.5 ± 2.5*	
Test group 2	Compound (I) 25 µg/mℓ	13.5 ± 2.5*	
Test group 3	Compound (II) 10 µg/mℓ		15.9 ± 1.3**

* : A significant difference from the control group ($P<0.001$)

**: A significant difference from the control group ($P<0.002$)

Test Example 4

Inhibiting activity of 7,4'-dihydroxy-isoflavone to the bone resorption potentiating action of parathyroid hormone in rat fetal long bone culture.

The bone samples prepared in the same manner as Test Example 3 were pre-incubated for 24 hours in the same medium as that prepared in Test Example 3 which contains bovine serum album in BGJ_b medium (Fitton-Jackson modification). Then, in the concomitant presence of PTH (parathyroid hormone, a bone resorption stimulant) and compound (II), the samples

were further incubated for 3 days and the percentage of ^{45}Ca released into the medium was calculated by means of the same formula as that in Test Example 3. The results are shown in Table 4. As control experiments, the same determination was made for a control group using the medium supplemented with PTH alone. It is apparent from Table 4 that compound (II) suppressed PTH-stimulated bone resorption.

Table 4

	Concentration of compound (II)	^{45}Ca (%) released
Control group	0	30.8±4.3
Test group	10 μg/mℓ	23.5±3.4*

*: A significant difference from the control group ($P<0.01$)

Test Example 5

Acute toxicity

Five-week-old ICR mice and 5-week-old Sprague-Dawley rats were respectively used in groups of 10 males and 10 females, and suspensions of compound (I) or compound (II) in olive oil were administered orally [2,500, 5,000 and 10,000 mg/kg of each compound] or subcutaneously [1,250, 2,500 and 5,000 mg/kg]. The animals were kept under observation for 14 days. None of the groups showed deaths nor toxic symptoms attributable to compound (I) or (II) and, therefore, LD_{50} values could not be calculated.

The daily dosage of the compound of the formula (I) according to this invention for human beings is generally about 1 to 50 mg/kg and preferably about 5 to 20 mg/kg for oral administration, and about 200 to 600 mg can be orally taken daily, once a day or, if necessary, in 2 to 3 divided doses. The compounds are preferably formulated into such dosage forms as tablets, capsules, etc. by the established pharmaceutical procedure. Such tablets and capsules can

be prepared using suitable excipients such as lactose, starch, etc., binders such as hydroxypropylcellulose, and lubricants such as magnesium stearate. The tablets may be sugar-coated, if necessary.

The following preparation examples are given to illustrate the invention in further detail and should not be construed as limiting the scope of the invention.

Example 1 Tablets

I)	7-Hydroxy-isoflavone	200 g
II)	Lactose	15 g
III)	Starch	44 g
IV)	Carboxymethylcellulose	10 g
V)	Magnesium stearate	1 g

The above components I) through V) were admixed to prepare 1000 uncoated tablets with a diameter of 8.5 mm.

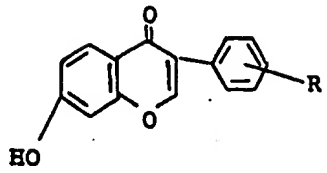
Example 2 Capsules

I)	7,4'-Dihydroxy-isoflavone	200 g
II)	Lactose	40 g
III)	Starch	50 g
IV)	Hydroxypropylcellulose	7 g
V)	Magnesium stearate	3 g

The above components I) through V) were admixed and filled into 1000 No. 1 capsules.

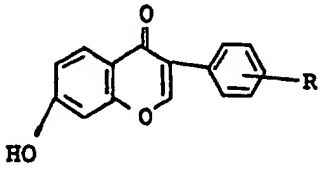
What is claimed is:

1. A compound of the formula



wherein R is a hydrogen atom or a hydroxy group for use in prevention or treatment of osteoporosis.

2. A pharmaceutical composition for prevention or treatment of osteoporosis, which contains an effective amount of a compound of the formula



wherein R is a hydrogen atom or a hydroxy group and a pharmaceutical acceptable carrier, vehicle, lubricant or diluent therefor.

3. A pharmaceutical composition according to claim 2, which is in the form of tablet, capsule, granule, fine granule, powder or syrup.

4 A pharmaceutical composition according to claim 2, wherein the osteoporosis is that caused by decreasing secretion of estrogen due to hypoovarianism.

D4

IMMUNO-SUPPRESSOR

Veröffentlichungsnr. (Sek.)	JP62106016
Veröffentlichungsdatum :	1987-05-16
Erfinder :	WATANABE SHUNICHI; others: 03
Anmelder :	YAMANOUCHI PHARMACEUT CO LTD; others: 01
Veröffentlichungsnummer :	⌜ JP62106016
Aktenzeichen: (EPIDOS-INPADOC-normiert)	JP19850245508 19851101
Prioritätsaktenzeichen: (EPIDOS-INPADOC-normiert)	
Klassifikationssymbol (IPC) :	A61K31/35
Klassifikationssymbol (EC) :	
Korrespondierende Patentschriften	

Bibliographische Daten

PURPOSE:To provide an immuno-suppressor containing a specific isoflavone compound as an active component, having low toxicity and excellent immuno- suppressing activity and useful for the remedy and the prevention of relapse of autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, etc.
CONSTITUTION:The isoflavone compound of formula (R<1> is OH or methoxy; R<2> is H, carboxyl or ethoxycarbonyl) is used as an immuno-suppressing agent. Concrete examples of the compound are 5,7,4'-trihydroxyisoflavone, 5,7- dihydroxy-4'-methoxyisoflavone-2-carboxylic acid, etc. The compound of formula has excellent immuno-suppressing activity and is useful for the remedy and prevention of relapse of human autoimmune diseases such as chronic active hepatitis, osteoporosis, etc. It is administered orally or parenterally at a dose of usually 200-1,000mg/day.

D5

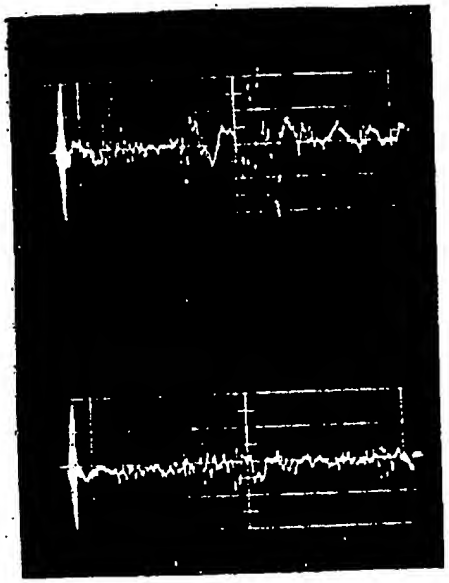


FIG. 1. Oscilloscope patterns of noises of internal infestation in wheat grains.

and removable cover 2½ in. thick, within which boxes of copper and Celotex surrounded the infested grain lying on the microphone. Records of wave patterns were made with a Tektronix oscilloscope and a Polaroid-Land camera attachment. Insects in the larval, pupal, and adult stages can be detected within the infested kernels, although the egg and extremely early stages of larval growth cannot. It appears that the larva must be nearly a week old before sufficient noise is produced for detection. In this research the stage of the infestation hidden within the grain kernels could be selected for study by means of x-ray techniques previously developed at this station (*3*).

Two distinct types of sound are associated with the larval and pupal stages, namely, a low-frequency scraping noise and a high-frequency tearing or rasping sound. From repeated observations it has been deduced that the low-frequency sounds are made by the movement of larva and pupa within the kernels, and the high-frequency sounds by the chewing of the endosperm of the grain by the larva. When several infested kernels are placed on the microphone, combinations of these frequencies may appear as shown in Fig. 1. Figure 1 was taken with the oscilloscope set at a sweep frequency of about 30 cps, and the insect sounds of both high and low frequencies are present. Thus in the upper trace the right and left ends show the low noise frequencies centering around 200 cps, characteristic of insect movement. Just to the right of the vertical scale in the center appears a high frequency burst of sound in the range of 1200–1500 cps. The frequency range of sounds due to internal insects appears to range from 200 to 8000 cps, although the lower limit has not been accurately determined. The voracious eating habits of the larval stage of rice weevil (*Sitophilus oryza* L.) has been clearly confirmed by this technique. It was also of interest to learn that, when the infested grains are disturbed in any way, the high-frequency sounds indicative of chewing usually cease and the low-frequency sounds, due apparently to movement, continue intermittently. After a short time the high-frequency sounds reappear. An experienced observer can estimate the approximate stage of development of the insect because the sounds are slightly different in the larval and pupal stages. This observation has suggested analysis of the recorded sound wave patterns as a means for differentiating developmental stages as well as physiological activities. Additional studies now in progress include evaluation of the method to determine numbers of infested kernels on the basis of cumulative recording of wave peaks, analysis of differences in sound characteristics of different species which infest grain internally, relationship of frequency of feeding and movement to stage of insect development, and determination of the influence of storage temperature and humidity on the nature, frequency, and periodicity of the sound patterns produced.

One practical application of this work is a means for the rapid evaluation of the effectiveness of fumigants whereby the normal delay of several weeks required for emergence of surviving insects, now necessary to determine fumigant efficiency, can be eliminated. A suitable sound detection device for performing such tests in mills and grain elevators is now under construction. Another application of the principle would be a means for monitoring grain within storage bins for infestation without sampling or removing the grain from the bins, in much the same manner as permanent thermocouple systems are now used for checking the heating of grain in storage.

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Estrogenic Activity of Isoflavone Derivatives Extracted and Prepared from Soybean Oil Meal[1]

Edmund Cheng, Charles D. Story, Lester Yoder, W. H. Hale, and Wise Burroughs

Iowa Agricultural Experiment Station, Ames

Implantation of diethylstilbestrol (synthetic estrogenic substance) pellets under the skin of cattle and sheep stimulates live-weight gains and increased feed

[1] Journal Paper No. J-2202 of the Iowa Agricultural Experiment Station, Ames, Iowa Project 1208.

efficiency during the fattening period (*1–3*). Recently, naturally occurring estrogenic substances have been detected in varying amounts in various plant herbages, both pasture and hays, fed to cattle and sheep (*4–6*). Genistein (5,7,4′-trihydroxyisoflavone) has been suggested by Curnow and Bennetts as the chemical constituent responsible for estrogenic activity in one of these herbages, namely, subterranean clover (*7*). The glucoside of genistein, genistin (5,4′-dihydroxy-7-glucosidoisoflavone) has been shown by Walter (*8*) to be present in substantial amounts (0.1%) in soybean oil meal. Since soybean oil meal is used rather widely in livestock feeding, it appeared advisable to investigate the estrogenic activity of both genistin and genistein in ascertaining their probable significance in livestock feeding.

Commercial soybean oil meal (solvent process) was extracted with methanol according to the method of Walter (*8*). Genistin was isolated as pale yellow, thin rectangular plates having a melting point of 256° C. Upon hydrolysis of genistin with hydrochloric acid in methanol, genistein was obtained. It was crystallized from hot 60% ethanol as white rectangular rods having a melting point of 298° C.

The estrogenic activity of these compounds was determined by the mouse uterine weight method described in detail in an earlier paper (*6*). The chemicals under study were either fed directly to immature female mice or were injected subcutaneously. Since neither genistin nor genistein is soluble in water, they were injected as their sodium salts. Six mice were used in each group, and the treatments were given once daily for 4 days. The mice were sacrificed 24 hr after the last treatment, their uteri dissected, fixed in Bouin's fluid, and weighed. The results obtained are presented in Table 1.

Feeding 2.5 and 5.0 mg of either genistin or genistein per day per mouse resulted in increased uterine weights. Injecting genistein at 1- and 2-mg levels respectively also increased uterine weights consistently over the corresponding weights of control animals. Whereas the injection of 1 mg of genistin did not have a measurable effect, the injection of 2 mg proved quite effective. It should be noted that these responses are similar to those due to the injection of 0.02–0.04 µg respectively of diethylstilbestrol, as shown in Table 1. The estrogenic activity of genistein can accordingly be estimated as approximately equivalent to 1/50,000 the activity of diethylstilbestrol. Genistin activity on a weight basis was slightly lower than that of genistein. However, the two compounds appeared to have approximately equal activity on a molecular basis.

Experiments are in progress with fattening lambs to determine whether the estrogenic activity of genistin as found in soybean oil meal is as beneficial as the estrogenic activity of stilbestrol, which has been shown experimentally to be valuable in lamb feeding. Although the estrogenic activity of genistin per unit of weight is small compared with that of diethylstilbestrol, the relatively large amount of genistin present in soybean oil meal, coupled with its presence in small amounts in certain hays, suggests the likelihood that the amounts present in certain cattle and sheep rations may be sufficiently large to exert major beneficial influences.

TABLE 1

ESTROGENIC ACTIVITY OF GENISTIN AND GENISTEIN

Group	No. of mice	Treatment[a]	Av uterine weight, mg
1	6	Normal control	9.7 ± 2.8
2	6	Feeding genistin, 2.5 mg	12.9 ± 4.4
3	6	Feeding genistin, 5.0 mg	39.8 ± 8.9
4	6	Injecting genistin, 1 mg	9.2 ± 1.7
5	6	Injecting genistin, 2 mg	14.6 ± 3.6
6	6	Feeding genistein, 2.5 mg	21.6 ± 13.4
7	6	Feeding genistein, 5.0 mg	23.6 ± 4.6
8	6	Injecting genistein, 1 mg	13.2 ± 2.4
9	6	Injecting genistein, 2 mg	17.0 ± 7.3
10	6	Injecting stilbestrol, 0.02 µg	13.2 ± 2.6
11	5	Injecting stilbestrol, 0.04 µg	18.8 ± 6.9

[a] Treatment was given daily for 4 days.

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The Reduction by Reactivating Light of the Frequency of Phenocopies Induced by Ultraviolet Light in *Drosophila melanogaster*

Max Perlitsh and Albert Kelner

Brandeis University, Waltham, Massachusetts

Phenocopies (*1*), or abnormalities of adults simulating mutations, are readily induced in *Drosophila melanogaster* by irradiating eggs, larvae, or pupae with ultraviolet light (*ca.* 2600 Å) (*2, 3*). Visible and near-ultraviolet light (3600–4900 Å) prevents ultraviolet-induced killing or mutation in bacteria and other organisms (*4, 5*). Such reversal of ultraviolet effects has been found in various organisms (see [*5*] for references) including, recently, *Drosophila*, where reactivating light lowered the incidence of ultraviolet-induced lethal mutations (*6*).

It became of interest to determine whether phenocopies induced by ultraviolet light in *Drosophila* were also affected by reactivating light, especially since the induction of phenocopies was perhaps a more complex phenomenon than those studied before.

D6

Therapie des klimakterischen Syndroms

von
Rainer Schrage

edition medizin

VCH

Prof. Dr. med. Rainer Schrage
Universitäts-Frauenklinik
Schleichstr. 4
D-7400 Tübingen

In diesem Buch enthaltene Dosierungsangaben wurden mit aller Sorgfalt überprüft. Dennoch übernehmen Autoren und Verlag – auch im Hinblick auf mögliche Druckfehler – keine Gewähr für die Richtigkeit. Dem Leser wird empfohlen, sich vor einer Medikation in jedem Fall über Indikationen, Kontraindikationen und Dosierung anhand des Beipackzettels oder anderer Unterlagen des Herstellers zu unterrichten. Das gilt insbesondere bei selten verwendeten oder neu auf den Markt gekommenen Präparaten.

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3.1 Typische Beschwerden und Veränderungen

Die hormonale Umstellung im Klimakterium, besonders der Östrogenmangel, bedingen eine Reihe von Beschwerden und klinischen Veränderungen. Andererseits beginnen sich in dieser Zeit auch die Alterungsvorgänge deutlicher auszuwirken, psychosoziale und kulturelle Faktoren beeinflussen ebenfalls das Beschwerdebild, so daß eine Trennung zwischen klimakterisch- und altersbedingten Veränderungen nicht immer möglich und teilweise auch umstritten ist. Die folgende Aufstellung (in Anlehnung an KOPERA 1974) soll daher nur eine Übersicht über die Beschwerden und Veränderungen dieser Zeit, weniger aber eine Aussage über deren Ursachen sein.

1. Blutungs- und Zyklusstörungen
   - Prä- und postmenstruelles Schmieren, Menorrhagien
   - Poly- und Oligomenorrhöen
   - Dysfunktionelle Blutungen

2. Vegetatives Syndrom
   - Hitzewallungen, (nächtliche) Schweißausbrüche
   - Rötungen der Haut
     oft damit einhergehend:
   - Schlafstörungen
   - Kopfschmerzen, Schwindelanfälle, Parästhesien
   - Herzbeschwerden, Herzklopfen, paroxysmale Tachykardien
   - Blutdruckkrisen
   - Periphere Durchblutungsstörungen
   - Meteorismus, Obstipation
   - Miktionsstörungen
   - Dyskinesien der Gallenwege
   - Rheumatoide Beschwerden, Gelenkschmerzen

3. Psychische Veränderungen
   - Energieverlust, Konzentrationsschwäche
   - Reizbarkeit, Aggressivität, Stimmungslabilität
   - Depressionen
   - Frustrations- und Versagensgefühle
   - Introversion, Verlassenheitsgefühle, antisoziales Verhalten
   - Karzinophobie

4. Organisches Postmenopausensyndrom
   - Atrophisch-entzündliche Veränderungen an Vagina, Urethra und Trigonum vesicae mit Dyspareunie, Dysurie und Harninkontinenz

- Kraurosis vulvae, Pruritus vulvae
- Atrophie der Haut, der Mammae
- Atrophische Stomatitis und Rhinitis

5. Metabolisches Postmenopausensyndrom
   - Osteoporose
   - Neigung zur Gewichtszunahme
   - Anstieg von Cholesterin
   - Atherosklerose, Koronarsklerose, Hypertonie, Herzinfarkt
   - Eventuell Neigung zu Diabetes mellitus, Gicht, Gelenkerkrankungen

11 Behandlung klimakterischer Ausfallserscheinungen mit pflanzlichen Wirkstoffen

In den letzten 10-20 Jahren ist sich der Patient zunehmend bewußt geworden, daß eine Therapie mit unerwünschten Nebenwirkungen und Komplikationen einhergehen kann. Medikamente werden daher mit Skepsis betrachtet und nicht selten einfach nicht eingenommen; besonders die „Hormone" werden verdächtigt, schwer vorhersehbare Risiken zu provozieren. Auch wenn es gelingt, einer Frau die hormonell bedingte Ursache der klimakterischen Beschwerden verständlich zu machen, kann dennoch eine kritische, wenn nicht gar ablehnende Haltung bestehen bleiben. Nicht nur publizistisch aufgemachte Berichte über Medikamentenfolgen, besonders während der Schwangerschaft, sind Grund dieser Zurückhaltung; wahrscheinlich besteht auch eine gewisse Analogie zur sog. Pillenmüdigkeit, da ja nun schon wieder einer Frau nahegelegt wird, einen bestimmten körperlichen und seelischen Zustand durch ständige Einnahme von Sexualhormonen zu erreichen und aufrechtzuerhalten. Die klimakterischen Beschwerden sind nicht allein durch Änderungen der Hormonspiegel bedingt, sondern auch durch psychische, soziale und kulturelle Faktoren geprägt. Sie unterliegen somit einer stark subjektiven Bewertung, die auch die therapeutischen Überlegungen tangiert, besonders, wenn eine Frau einer Hormonbehandlung kritisch gegenübersteht. Es ist durchaus möglich, daß die Patientin dem Arzt gegenüber die klimakterischen Beschwerden verschweigt oder bagatellisiert, um nicht mit dem Vorschlag einer Hormonbehandlung konfrontiert zu werden. Bei aller Berechtigung, den Patientinnen eine solche Behandlungsmöglichkeit positiv vor Augen zu führen, sollte eben auch diese kritische Einstellung vom Arzt bedacht und respektiert werden.

Klimakterisch bedingte Beschwerden mit pflanzlichen Wirkstoffen anzugehen, bedeutet einen Ausweg, der nicht nur bei Kontraindikationen der Hormontherapie Interesse verdient. Dabei kann man bei Verwendung pflanzlicher Präparate drei verschiedene Wirkungsstufen unterscheiden. Bei der einen oder anderen Patientin im Klimakterium kann es berechtigt sein, ein Präparat im Sinne eines Plazebos einzusetzen. Eine nächst höhere Wirkungsstufe stellt die sog. Pseudoplazebotherapie dar; schließlich können pflanzliche Präparate angewandt werden, die Substanzen enthalten, die in ihrer Wirkung z.B. den chemisch definierten Östrogenen entsprechen.

1. Pflanzliche Präparate werden gern und häufig Plazebos gleichgestellt. Selbstverständlich kann in dem einen oder andern Fall allein der Plazeboeffekt ausschlaggebend sein. Auch die Plazebotherapie stellt aber einen gangbaren und vertretbaren Weg dar, wenn wirksamere bzw. kausal wirkende Mittel fehlen oder sich verbieten. Wenn auch bei den Beschwerden in den Wechseljahren die psychische Komponente eine wesentliche Rolle spielt, sind in diesen Jahren Art und Weise, Konflikte zu bewältigen, doch zumeist festgelegt und festgefahren, und diesbezüglich neue Wege lassen sich nur unter großen Anstrengungen von Patientin und Arzt erarbeiten. Eine psychotherapeutische Behandlung ist also meist langwierig und wird von einer Frau häufig auch gar nicht gewünscht. Eine Plazebotherapie mit pflanzlichen Präparaten – mit pflanzlichen, weil hier das Mißtrauen noch am geringsten ist und Nebenwirkungen sehr selten sind – kann daher gerechtfertigt sein, wenn die Indikation individuell gestellt ist, eine Hormonbehandlung abgelehnt wird oder Kontraindikationen dagegen sprechen. Natürlich ist es möglich, daß der Plazeboeffekt nicht lange anhält und ein neues Überdenken der Behandlung erforderlich macht.

2. Nun würde man auch bei der Behandlung klimakterischer Beschwerden die Phytotherapie unterschätzten, wenn ihr nur ein Plazeboeffekt eingeräumt werden sollte. Hinsichtlich ihrer Wirkung ist über die Plazebotherapie die sog. Pseudoplazebotherapie zu stellen. Hierunter ist die Anwendung von Substanzen zu verstehen, die an und für sich biologisch wirksam sind, jedoch nicht im Sinne der Indikation, oder aber die in einer zu niedrigen Dosis verabreicht werden. Es kann sich hier um chemisch definierte Stoffe, aber auch um pflanzliche handeln. Es ist, wie HÄNSEL (1980) betont, besser, anstelle eines hochwirksamen Arzneimittels ein „mildes" Phytopharmakon als Pseudoplazebo einzusetzen, da dieses nur ein geringes therapeutisches Risiko in sich birgt.

3. Pflanzliche Arzneimittel haben häufig eine „milde" Wirkung. Damit ist gemeint, daß ihre maximale Wirkungsstärke relativ gering, ihre therapeutische Breite groß ist, toxische Nebenwirkungen fehlen und ein Wirkungseintritt erst nach längerer Anwendungszeit zu beobachten ist. Aufgrund dieser Eigenschaften ordnen sich milde Phytopharmaka zwanglos am Anfang der therapeutischen Skala ein, da eine Dosissteigerung über die maximale Wirkungsstärke keine weitere Wirkungssteigerung hervorruft; bei Bedarf ergibt sich dann die Indikation für ein hochwirksames Arzneimittel. Die große therapeutische Breite der Phytopharmaka ist ein weiterer Vorteil; eine schädliche Dosis kann aus Mengengründen meist gar nicht erreicht werden. Schließlich ist zu bedenken, daß die Wirkung von Phytopharmaka auf andere Weise zustande kommt als die der entsprechenden chemisch definierten Mittel; deren Dosen und Nebenwirkungen lassen sich daher bei gleichzeitiger Anwendung pflanzlicher Mittel reduzieren.

Bei den zur Behandlung klimakterischer Beschwerden empfohlenen Phytopharmaka sind zwei Wirkungsrichtungen von Interesse: die östrogene und die

sedierende. In vielen Pflanzen konnten östrogene Wirkstoffe nachgewiesen werden. Die wirksamen Substanzen, soweit sie identifiziert wurden, unterscheiden sich chemisch erheblich von den Östrogenen des Organismus. Einige haben eine gewisse Ähnlichkeit mit den synthetisch hergestellten Stilbenen; sie sind jedoch in ihrer Wirkung schwächer als die Stilbene und natürlichen Östrogene. Die Wirkungsstärke der „pflanzlichen Hormone" kann aber ausreichend sein. Ein eigener Wirkungsmechanismus, der sich von dem der körpereigenen Östrogene unterscheidet, ist anzunehmen. Mit „pflanzlichen Sexualhormonen" läßt sich eine Proliferation des Vaginalepithels erreichen, und auch das Vegetativum wird beeinflußt: östrogenwirksame Pflanzenhormone haben einen parasympathiko-, gestagenwirksame einen sympathikomimetischen Effekt.

Die sedierenden Substanzen der Phytopharmaka sind in ihrer chemischen Struktur weitgehend unbekannt. Ihre zentral dämpfenden Eigenschaften sind aber erwiesen und gehen mit Sicherheit über eine Plazebowirkung hinaus. Der Vorteil dieser milden Phytopharmaka besteht vor allem darin, daß sie kaum unerwünschte Nebenwirkungen aufweisen. Da auch ihre maximale Wirkungsstärke niedrig liegt, können je nach Situation hochwirksame chemisch definierte Sedativa bzw. Hypnotika mit den bekannten Nebenwirkungen in beträchtlichem Umfang eingespart werden. Durch Kombinationen verschiedener Phytopharmaka, auch mit geringen Mengen Phenobarbital, lassen sich gerade klimakterische Beschwerden günstig beeinflussen, indem sowohl der Tonus des Sympathikus wie auch der des Parasympathikus gedämpft wird, die extremen Tonusschwankungen des Vegetativums somit gemildert werden; man spricht daher von „Zugleichmitteln".

Im folgenden werden nun pflanzliche Wirkstoffe behandelt, die allein oder in verschiedenen Kombinationen zur Behandlung klimakterischer Ausfallserscheinungen dienen. In der Liste der Pharmaka sind sie als Fertigarzneimittel angeführt (S. 140).

11.1 Cimicifuga racemosa (Schlangenkraut, Wanzenkraut, Traubensilberkerze)

Die Pflanze kommt in den Wäldern Nordamerikas und Kanadas als etwa 30-60-100 cm großes Hahnenfußgewächs vor. Arzneilich werden die Wurzeln verwendet, die bis zu 12 cm lang und etwa 2,5 cm dick werden. Bei den Wirkstoffen handelt es sich u.a. um Triterpenglykoside, wie Cimicifugosid, Cimigenol, Aktein und Akteol. Außerdem sind hier Phytosterin, Gerbstoffe, ätherische Öle und eine Reihe anderer Substanzen enthalten (HARNISCHFEGER und STOLZE 1980, 1983). Wahrscheinlich sind es die Triterpenglykoside, die die vasomotorischen Zentren hauptsächlich beeinflussen. In einer tierexperimentellen Untersuchung (ovarektomierte Ratte) zur endokrinen Wirksamkeit der Inhaltsstoffe von Cimicifuga racemosa konnten JARRY und

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V/1 Occurrence of Anabolic Agents in Plants and their Importance

D7

H.R. Lindner
Department of Hormone Research, The Weizmann Institute of Science, Rehovot, Israel

Summary

More than 40 plant species have been shown to contain substances that are active in biological assays for estrogenic activity. Such substances may be constitutive metabolic products of a plant, or be formed adaptively in response to environmental factors, such as fungal attack (e.g. coumestrol synthesis in alfalfa infected with *Pseudopeziza medicagensis*); in other instances estrogens may arise from microbial attack on plant material during storage (e.g. zearalenone formation from corn by *Fusarium* spp.). Phyto-estrogens may reach man through direct consumption of fresh fruit, vegetables and processed plant products (e.g. administration of olive or cornoil can induce vaginal keratinization in post-menopausal women); or – more relevant to this Symposium – by consumption of carcasses and products from animals fed estrogen-containing forage.

Important pasture and forage plants shown to contain phyto-oestrogens include *Trifolium subterraneum L.*, notably the cultivars Dwalganup, Mt. Barker, Yarloop and Marrar, *T. pratense* (red clover), *T. fragiferum L.* (strawberry clover), *T. alexandrinum* (berseem clover), *Medicago sativa* (alfalfa or lucerne) and *Soya hispida* (soya beans). A beneficial anabolic action of the estrogens contained in these plants has been implied, but not unequivically established. More attention has been paid to their noxious effects on livestock. On affected *T. subterraneum* pasture, castrated male sheep showed lactation, squamous metaplasia of the bulbo-urethral glands and urethral stenosis; infertility, variously attributed to suppression of gonadotrophin release and ovulation; faulty ovum transport; premature regression of corpora lutea; irreversible cystic hyperplasia of endometrial glands on prolonged exposure; dystocia and prolapse of the uterus. Sporadic incidence of phyto-estrogen induced infertility in cattle has been reported, attended by ovarian cyst formation. Estrogenic activity in forage plants has been reported from Australia, New Zealand, India, Sweden, Great Britain, Germany, Denmark, Holland, Finland, Egypt and Israel.

The clover constituents chiefly incriminated for these effects are glycosides of the isoflavone derivatives genistein and its 4'-methyl ether biochanin-A, daidzein and its 4'-methyl ether formononetin, and pratensein; coumestrol and its 3'- and 4'-methyl ethers account for the estrogenic activity of alfalfa. The isoflavone content of subterranean clover may reach 3 percent of its dry weight, and the coumestrol content of lucerne may exceed 100 µg/g. Coumestrol and genistein compete with 17β-estradiol for binding sites on the uterine cytoplasmic receptor and induce macromolecular synthesis in the uterus, but fail to induce ovum implantation in ovariectomized, gestagen-maintained rats. Uterotrophic activity of coumestrol and genistein given parenterally to sheep is approximately 10^{-3} and 10^{-5} times that of stilboestrol, respectively. Biological activity of ingested phytoestrogens is modified by ruminal micro-organisms and hepatic metabolism. The pro-estrogens 4'-methylcoumestrol, biochanin-A and formononetin undergo *O*-demethylation in the rumen and liver to give rise to coumestrol, genistein and daidzein, respectively. Daidzein is further metabolized in the rumen to equol (about 70 percent) and *O*-desmethylangolensin (5–20 percent), both of which possess weak but significant estrogenic activity; genistein and biochanin-A are transformed chiefly to hormonally inert *p*-ethylphenol. The greater part of the circulating phyto-estrogens occur as glucuronide conjugates. Limited data indicate that estrogenic isoflavones and coumestans accumulate in fat depots in sheep grazing affected pasture, but the amounts reported (about 1 p.p.m.) seem too low to present a significant

health hazard to the human consumer. However, no information on the pharmacology of these substances in primates is available.

In addition to biological screening techniques, specific methods have been developed for chromatographic separation of phyto-estrogens and their determination by spectrophotometry and fluorometry, receptor radioassay or radioimmunoassay. Control measures under investigation include pasture management, selection of isoflavone-deficient mutants and active vaccination with synthetic isoflavone derivatives coupled covalently to polypeptide carriers.

1. Introduction: ecological considerations

Close to 50 plant species have by now been shown to contain substances that are active in biological assays for estrogenic activity conducted in laboratory rodents or in ruminants (for reviews see refs. 10, 15, 46, 79). Such substances may be constitutive metabolic products of the plant, or be formed adaptively in response to environmental factors, such as fungal attack. Thus coumestrol synthesis is induced or greatly augmented in alfalfa (*Medicago sativa*) on infection with the leaf-spotting organism *Pseudopeziza medicagensis*[12,70]. In other instances estrogens may arise from microbial attack during faulty storage, e.g. zearalenone is formed in corn contaminated with *Fusarium spp.*[19]. The ecological factors that determine the balance between estrogenic and non-estrogenic species in natural pastures and the remarkable seasonal and geographical variation in the estrogen content of a given clover strain have not been fully identified[10]. They include among others, the level of phosphate fertilizer applied[3,62].

Oestrogenic activity may occur in bulbs (e.g. *Allium sativum L.*[30] or tubers (e.g. *Butea superba Roxb.*[64], or be localized in the leafy parts of the plant, in its fruit or in its seed[15]. Such phyto-estrogens may reach man through direct consumption of fresh fruit, such as apples[63] and cherries[27,63], vegetables (e.g. potatoes[15]) or condiments such as garlic[30]; from hops used for beer production[90] and other processed plant products; or by consumption of carcasses and products from animals fed estrogen-containing forage. There is no published evidence that herbal estrogens reaching the human from any of these sources is of pathogenic significance. It is of interest, however, that administration of corn oil or olive oil, at the rate of 100 g per day over 10 days, was shown to cause extensive keratinization of the vaginal epithelium in post-menopausal women[84], indicating that herbal estrogens contained in these products are indeed biologically active in man and effectively absorbed from the gut.

2. Distribution and chemical nature

Important pasture and forage plants shown to contain phyto-oestrogens include *Trifolium subterraneum L.*, notably the cultivars of Dwalganup, Mt. Barker, Yarloop, Clare, Geraldton, Dinninup, Woogenellup and Marrar [8,9,13,14,25,41,43], *T. pratense* (red clover[40,87,88]), *T. fragiferum L.* (strawberry clover[61]), *T. alexandrium* (berseem clover[35,68,69]), *T. repens* (Ladino clover[55]), M. sativa (alfalfa or lucerne[11,70] and *Soya hispida* (soya beans[20,69,86]). Only in very few cases were plant estrogens found to be identical with one of the estrogenic hormones of mammals. Examples are the isolation of estrone from palm kernels by Butenandt[18], and the reported occurrence of estriol in willow catkins[78]. With these esoteric exceptions, however, the phytoestrogens thus far identified proved to be phenols not chemically related to the hormonal steroids, but they share with 17β-estradiol certain structural features (Fig. 1) that may account for their biological activity.

The clover constituents chiefly associated with estrogenic activity are glycosides[8] of the isoflavone derivatives genistein[14] and its 4'-methyl ether biochanin-A[58], daidzein and its 4'-methyl ether formononetin (Fig. 2; ref.[13,16,41,43,44,72]) and pratensein[88]; coumestrol

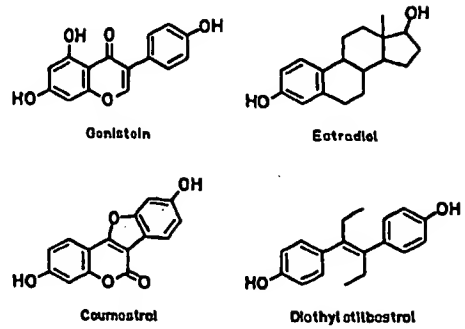

Fig. 1. Structural similarity between non-steroidal estrogens and estradiol

and its 3'- and 4'-methyl ethers account for the estrogenic activity of alfalfa and medics[10,11,16,70,75]. The isoflavon content of subterranean clover may reach 3% of its dry weight[41] and the coumestrol content of lucerne may exceed 100 μg/g[10,69]. The uterotrophic activity of coumestrol and genistein given parenterally to sheep is approximately 10^{-3} and 10^{-5} times that of stilbestrol respectively[16]. Both compounds were also active by the intraruminal route, but potency was 1/20 (genistein) to 1/100 (coumestrol) of that observed on intramuscular administration of the same compound[16,41].

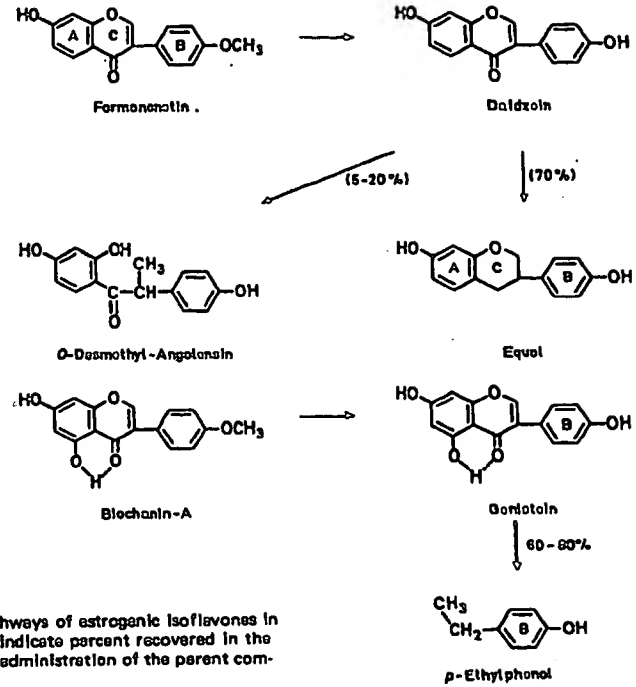

Fig. 2. Major metabolic pathways of estrogenic isoflavones in sheep. Numbers in brackets indicate percent recovered in the urine following intraruminal administration of the parent compound (ref.[72])

A recent analysis of five samples of soybean oil cake (Shemesh, Ayalon and Lindner, unpublished observations), an important ingredient of dairy-cattle diets, showed the presence (p.p.m. in dry matter ± S.E.M.) of daidzein (30.0 ± 4.7), formononetin (4.3 ± 0.2), genistein (18.6 ± 2.7), coumestrol (16.5 ± 2.9) and 4'-methylcoumestrol (0.3 ± 0.02). Berseem clover (*T. alexandrium*), which plays an important part as a cattle feed in the Middle East, was shown by bioassay to contain estrogenic activity[35,68]). Chemical analysis proved that it contains the isoflavones genistein, biochanin-A and formononetin, as well as the coumestan derivative coumestrol (Shemesh, Ayalon and Lindner, unpublished observation); the plasma of heifers fed this forage contained, in addition, daidzein, probably an *O*-demethylation product of formononetin (v.i.).

3. Mode of action

The primary interaction of estradiol with its target cells appears to involve the binding of the hormone to a cytoplasmic protein receptor, characterized by a sedimentation constant of 8S in low-salt sucrose gradients. Both coumestrol and genistein compete with 17β-estradiol for binding sites on this receptor in uterine cytosol preparations from rabbit[71] or sheep[76], but the affinity of the phyto-estrogens is considerably below that of estradiol. The affinity of their methylated derivatives (e.g. formononetin and 4'-methylcoumestrol) for the receptor is still lower, though these compounds are estrogenically active *in vivo*. Isoflavan-7,4'-diol (equol) almost equals genistein in affinity for the receptor. The significance of this compound will be discussed later in relation to the metabolism of formononetin in ruminants.

Coumestrol and genistein also simulate estradiol in stimulating macromolecular synthesis in the uterus[51]. In particular, both phyto-estrogens stimulate the *de novo* synthesis of a specific "estrogen-induced protein", demonstrable within 1 h in the cytoplasm of the rat uterus by double-labelling techniques and gel-electrophoresis (A.M. Kaye, D. Sheratzky-Sömjen and H.R. Lindner, unpublished observations). This protein[52] is considered crucial for the action of estradiol[6]. While both coumestrol and genistein exert uterotrophic activity in the rat, neither compound is able to induce ovum implantation in ovariectomized gestagen-maintained rats, suggesting that the latter response involves a different receptor mechanism[57].

4. Metabolism in grazing animals: role as pro-estrogens

The biological activity of the ingested isoflavones and coumestans is modified by ruminal microorganisms and hepatic metabolism. 4'-Methylcoumestrol, biochanin-A and formononetin undergo *O*-demethylation in the rumen and liver to give rise to coumestrol, genistein and daidzein, respectively (Fig. 2, p. 159; ref.[41,50]). Daidzein is further metabolized in the rumen to equol (about 70%) and *O*-desmethylangolensin (5–20 %), both of which – notably equol – possess weak but significant estrogenic activity; genistein and biochanin-A are transformed chiefly to hormonally inert *p*-ethylphenol (Fig. 2; ref.[16, 72, 73, 75]). Formononetin is also reduced without prior demethylation to 4'-*O*-methyl-equol, which appears in the urine following administration of formononetin to sheep (Cox, Braden and Lightfoot, personal communication). The different metabolic patterns of the 5-hydroxy and 5-deoxy isoflavones probably account for the observation that the estrogenic effects of clover pastures on grazing sheep are more closely correlated with their formononetin than with their genistein content[43,44], in spite of the greater estrogenicity of genistein in parenteral assays in laboratory rodents and sheep[16]. Formononetin and the various methylated derivatives of genistein and coumestrol are thus properly classified as pro-estrogens.

The greater part of the circulating phyto-estrogens in sheep occur as water soluble glucuronide or sulphate conjugates[73].

5. Anabolic action

A beneficial anabolic action of plant estrogens on grazing animals has been implied, but not unequivocally established. The evidence for this view is, in the main, indirect: castrate lambs grew faster when fed estrogen-containing alfalfa, or when given alfalfa extracts containing coumestrol, than those raised on a non-estrogenic diet[56]. Intact female lambs did not show this response. Again, the growth response to stilboestrol is diminished or abolished in animals consuming estrogenic pasture. This was interpreted to indicate that such animals already receive maximal estrogen-mediated anabolic stimulation from their plant diet[31]. These reports are suggestive but not conclusive, if only because dietary factors other than the plant estrogens may have confounded the results. Equally inconclusive are reports attributing the so-called "spring flush" in milk yield to the estrogen content of pasture[4,5, 32,59], or the finding that the plant estrogen coumestrol enhanced the tenderness and juiciness of lambs as judged by a chewing panel[33].

6. Noxious effects on livestock

Much more attention has been paid to the noxious effects of phyto-estrogens on farm animals, and more critical work has been done in this area. This problem was first recognized in 1946 when Bennets and Underwood[9] described massive outbreaks of infertility in sheep grazing subterranean clover in Australia. Subsequently, genistein was isolated from this clover[14]. "Clover disease" is still regarded as one of the major problems of livestock production in Australia[42,45], with about 9 million sheep at risk, and in its milder form it has been recognized as a breeding problem in many other countries. Estrogenic activity in forage plants has been reported in New Zealand[48], the Phillipines[2], Japan[55], The United States of America[10], Canada[37], Chile[31], Central Africa[82], India[36], Egypt[60], Israel[1,70], The Union of Soviet Socialist Republics[77], Finland[34,81], Sweden[49], Denmark[47], Great Britain[59,60], Belgium[26], Holland[85], Germany[65,67], Italy[23] and Czechoslovakia[21,22].

The biological effects of clover estrogens responsible for fertility impairment appear to be multiple. Ewes exposed to affected subterranean clover pastures show mild to severe degrees of infertility, attributed to faulty sperm and ovum transport and interference with ovum implantation[46], premature regression of corpora lutea[53] or cystic hyperplasia of the endometrial glands, leading to irreversible sterility on prolonged exposure[9,46]. Other disturbances include maternal dystocia and prolapse of the uterus, often followed by gangrene in the field[9]. Phyto-estrogens may suppress gonadotrophin secretion[39], possibly by interfering with the positive feedback effect of endogenous estradiol on the hypothalamus (J. Goding, personal communication). Castrated male sheep grazing estrogenic *T. subterraneum* pastures showed lactation, squamous metaplasia of the male accessory glands, at times with gross enlargement of the bulbo-urethral glands and urinary retention[9,46]. The possibility that clover estrogens may cause seminal degeneration in rams has been considered on indirect evidence[46]. Sporadic incidence of phyto-estrogen-induced infertility in cattle has been reported, usually attended by ovarian cyst formation and occasionally by nymphomania[1,23,54,60,66,80, 83,89].

7. Residues in animal carcasses

Limited data[41] indicate that estrogenic isoflavones (genistein, biochanin-A, formononetin and daidzein) accumulate in fat depots in sheep grazing estrogenic pasture or given synthetic isoflavones by intra-ruminal infusion. The amounts found in the adipose tissue (about 1 p.p.m.) exceeded the concurrent plasma concentration. Nevertheless, the concentrations reported appear too low to present a serious health hazard to the human consumer, considering the low estrogenic potencies of these substances (cf. ref. 79). However, no infor-

ıation on the pharmacology of the herbal estrogens in primates is available. The possibi-
ty that such estrogens may pass into milk or milk products should also be kept in mind.

. Methods of detection

iological assays, using the uterine weight or vaginal cornification response in ovariectom-
ed laboratory rodents or ewes, or the teat growth response in castrated male sheep, have
een found useful for screening purposes[16,17,38,46]. The species used (ruminants vs. mono-
astric animals) and the route of administration (oral vs. parenteral) may markedly affect
ıe estimate of relative potency obtained, and due regard must also be paid to low solubi-
ty of the isoflavones and coumestans in neutral aqueous media.

aper- thin-layer and gas-chromatographic micromethods are available for the separation of
ll the known phyto-estrogens from plant material, body fluids and animal tissue[10,28,41,87]
ıd for their quantitative determination by spectrophotometry and fluorometry[10,41],
ame-ionization detection[41] or receptor radioassay[71]. Another feasible approach is radio-
imunoassay[7]. Receptor radioassay will not distinguish between the endogenous mamma-
an estrogens and nonsteroidal phyto-estrogens or stilboestrol, and will not detect the meth-
lated herbal pro-estrogens. Immune sera, generated with isoflavone or coumestan haptens
ovalently attached to a protein carrier, will discriminate between steroidal and isoflavone-
erived estrogens, and can be used to measure the active phenols as well as their *O*-methyl
hers[7].

Control measures

ontrol measures under investigation include pasture management designed to preserve a
vourable balance between estrogenic legumes and grass[3,10,46,62]; measures to limit fun-
l infection of forage plants[12]; selection of isoflavone deficient clover mutants[29], which
ıould have desirable agronomic properties, such as the ability to compete with the wild
pe in the field; and active vaccination with synthetic isoflavone derivatives coupled co-
lently to polypeptide carriers[7]. High titres of specific antibodies to phyto-estrogens are
aintained in sheep for more than a year after primary immunization, are transferred to
mbs through the colostrum and do not interfere with the action of endogenous hormones
breeding performance[20]. The protective value and economic feasibility of such vaccina-
ın remains to be established, and a more acceptable adjuvant than CFA, which is cur-
ntly used, may have to be found. If effective, this method would provide the first ex-
ıple of immunization against a naturally occuring disease with a fully synthetic antigen,
ıd as such could be of more general interest.

. Concluding remarks

trogenic substances are widely distributed among plants serving as animal fodder or
own for direct human consumption. By and large they are nonsteroidal compounds cap-
le of interacting directly with the cytoplasmic estrogen receptor by virtue of a structu-
l resemblance to estradiol, — or apt to be metabolized to such estrogenomimetic com-
ıunds. It is likely that these compounds possess anabolic activity, but this aspect needs
ore critical documentation, preferrably including trials with purified substances. In ex-
ssive amounts herbal estrogens clearly have adverse effects on reproductive performance
sheep, and more sporadically in other ruminants. Some control measures under active
vestigation were briefly discussed.

sidues of these estrogens may accumulate in the carcass, and methods are available for
eir detection, but there is no information to suggest that they present a serious health
zard to man. The ubiquitous background of herbal and endogenous estrogens in animal products may have to be considered when designing regulatory measures and forensic assay procedures to control the use of synthetic anabolic agents. Furthermore, the possible presence of phyto-estrogens in the control diet of experimental animals should be kept in mind when examining the effectiveness of other anabolic agents.

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V/2 Endogenous Anabolic Agents in Farm Animals

Weiert Velle

Department of Physiology, The Veterinary College of Norway, P.O. Box 8146 Dep., Oslo 1, Norway

Summary

This presentation is limited to the three groups of steroid sex hormones which alone or in combination have been shown to be anabolic when used in farm animals. It seems essential for realistic evaluation of public health aspects of use of these hormones that the discussions include *naturally occurring* levels of the hormones.

The following topics will be dealt with for each group of hormones: 1. Types and sources; 2. Production rates; 3. Plasma levels; 4. Tissue concentrations; 5. Metabolism and excretion.

Gestagens

Progesterone and 20-dihydroprogesterones are mainly produced in ovaries and placenta. Production rates are estimated to 10 and 14 mg/24 hrs in pregnant goats and sheep, respectively. Plasma levels during the luteal phase are of the order of 2—10 ng/ml, during pregnancy somewhat higher. Muscular tissue from calves contain 0.25 ng/g. In dairy cows progesterone is excreted with the milk which contains up to 30 ng/ml; butterfat up to 300 ng/ In ruminants progesterone is metabolized mainly to androgens excreted with faeces. In pigs large parts are metabolized to pregnanediols excreted with urine.

Androgens

Testosterone is mainly secreted by testes. Boar testes also produce large amounts of dehydroepiandrosterone and its sulphate. Production rates have been estimated to be 10 mg and 40—50 mg/24 hrs. in boars and bulls respectively. Plasma levels in bulls and rams are generally 2—10 ng/ml, in boars 2—25 ng/ml. Adipose tissue levels up to 22 ng/g are reported for bulls. In ruminants epitestosterone seems to be a major metabolite excreted mainly with faeces. In boars, urinary 11-deoxy-17-ketosteroids are major metabolites of testicular dehydroepiandrosterone. Castration shows elimination to be rapid.

Estrogens

17β-Estradiol and estrone are produced in ovaries and placenta and, in large amounts, in boar and stallion testes. Production rates in late pregnancy are estimated to 10 mg oestron 24 hrs. in goats, 2 mg estrone and up to 28 mg 17β-estradiol/24 hrs. in sheep. In cows mu higher values are found. Boars and stallions produce huge amounts daily. Plasma levels in non-pregnant animals are at the pg/ml level. In late pregnancy levels of 2—4 thousand pg/n are encountered in sows and cows, in sheep and goats lower levels. Calf muscular tissue co tains up to 410 and 610 pg/g of estrone and 17β-estradiol respectively. In muscle from pregnant heifers corresponding values were 120 and 860 pg/g in the 4th month and 2100 and 370 pg/g in the 9th month of pregnancy. Ruminants in large measure metabolize 17β-estradio and estrone to 17α-estradiol which posesses low estrogenic activity. In pigs estrone dominates in blood and urine. Major routes of elimination are with faeces in ruminants, with urine in pigs and horses. Elimination rates are high.

Results obtained during the last few years clearly show that all three groups of steroid sex hormones occur in considerable concentrations in plasma and tissues. Realization of claims of zero tolerance levels for these compounds is therefore impossible. The problems may be different when compounds other than those occurring naturally are considered.

D8

MEDICINAL PLANTS of CHINA

Vol. 1

James A. Duke
Edward S. Ayensu

REFERENCE PUBLICATIONS, INC.

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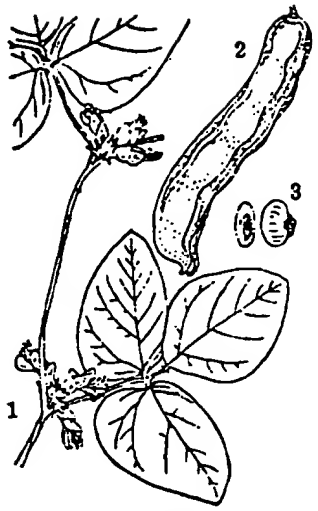
Stizolobium capitatum (Sweet) O.Ktze.
NAMES: Pinyin: lí dòu.
USES: *Plant:* Tonic. *Ref:* 16.
CHEM.: L-3,4-dihydroxyphenylalanine and mucunine. Hay from other species of *Stizolobium* contains (ZMB) per 100 g: 17.7-19.6% protein, 3.3-3.9% fat, 65.3-71.0% total carbohydrate, 28.5-29.6% fiber, and 8.0-11.2% ash. *Ref:* 16, 25, 148.

5491 黎豆 lí dòu (《本草拾遗》)

野决明
1.花枝 2.花 3.果实 4.种子



头花黎豆
1.花枝 2.果实 3.种子

Thermopsis lupinoides (L.) Link
NAMES: Pinyin: yě jué míng.
USES: *Fruit:* For diseases of mouth, teeth, and throat. *Ref:* 16, 35.
CHEM.: Contains cytisine, pachycarpine, d-sparteine, thermopsine. *Ref:* 25.

4377 野决明 yě jué míng (《高原中草药治疗手册》)

Trifolium pratense L.
NAMES: Pinyin: hóng chē zhóu cǎo. English: red clover.
USES: *Flower:* Decoction of flower heads expectorant. *Plant:* Anticancer. Regarded as alterative, antiscrofulous, antispasmodic, aperient, detergent, diuretic, expectorant, sedative, and tonic. Used for athlete's foot, burns, cancer, constipation, corns, gall-bladder, gout, liver, rheumatism, skin, sore eyes. *Ref:* 16, 25, 32, 38.

红车轴草
1.植株全形 2.花 3.果实

2049 红车轴草 hóng chē zhóu cǎo (《中国药植图鉴》)

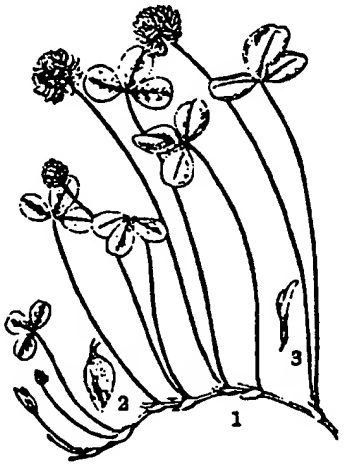
CHEM.: Estrogenic disorders have been reported in cattle grazing largely on red clover, apparently due to activity of the isoflavones formononetin, biochanin a, and to some small extent daidzein and genistein. The flowers contain a number of phenolic compounds: daidzein, genistein, isotrifolin, isorhamnetin, pratol, pratensol, trifolin, and an antifungal compound trifolirhizin. They also contain coumaric acid, hentriacontane, heptacosane, myricyl alcohol, and B-sitosterol. On a dry basis flowers yield 0.028% of an oil containing furfural. Seeds are reported to contain trypsin inhibitors and chymotrypsin inhibitors. Green forage of red clover is reported to contain: 81% moisture, 4.0% protein, 0.7% fat, 2.6% fiber, 2.0% ash. Hay of red clover contains 12.0% moisture, 11.8% protein, 2.6% fat, 27.2% fiber, and 6.4% ash. Hay contained (ZMB): 8.3-24.7% protein (avg. 14.9%), 1.0-6.6% fat (avg. 2.9%), 12.5-39.3% crude fiber (avg. 30.1%), 3.1-14.0% ash (avg. 7.9), and 33.4-59.1% N-free extract (avg. 44.2). *Ref:* 38.

Trifolium repens L.

NAMES: Pinyin: sān xiāo cǎo.
English: white clover.

USES: *Plant:* Said to be antirheumatic, antiscrofulous, depurative, detergent, tonic, and prophylactic for mumps. A tincture of the leaves is applied as an ointment in gout.

CHEM.: Seeds are reported to contain trypsin inhibitors and chymotrypsin inhibitors. The species is polymorphic for cyanogenic glucosides. Leaves and flowers of certain cyanogenic phenotypes contain a glucoside which releases cyanide on contact with the enzyme linamarase. All cvs have a low estrogen content (usually 6.3% for mononetin).The dominant estrogen is coumestrol. Late in the season diseased leaves may be more estrogenic than healthy leaves. Hay contains (ZMB): 13.1-32.4% crude protein (avg. 22.0%), 1.5-4.7% fat (avg. 2.6), 12.8-37.1% crude fiber (avg. 23.3), 4.7-14.8% ash (avg. 10.1), and 37.8-46.5% N-free extract (avg. 42.0). *Ref:* 38.



白车轴草
1.花枝 2.果实 3.花

0117三消草 sān xiāo cǎo (《贵州民间药物》)

D9

Herbal Help to Avoid Menopause Symptoms

by Nancy Beckham

The aim of this article is to provide information on non-harmful ways of overcoming the problems of menopause.

The information given may also be applicable to women who already have osteoporosis or for younger women who have had their ovaries removed, however these two categories of women should seek professional guidance.

SOME STATISTICS

Twenty-five per cent of women in the 45–55 age range have no menopausal symptoms. Of the 75 per cent who have problems, the following is a breakdown of the symptoms:

Flushing and sweats	80%
Lethargy	70%
Nervous problems such as anxiety, depression, irritability	70%
Reduced sex drive	65%
Insomnia	60%

Other symptoms include hair and skin changes, poor memory and lack of concentration, headaches, dry vagina, pain during intercourse, loss of confidence, loss of femininity and urinary symptoms. Of course, not all of these are necessarily linked to low oestrogen levels and could be related to dietary and lifestyle factors and the 'normal' aging process. After middle age, men also find they have less energy, a lower sex drive and generally sleep less.

Osteoporosis is the most serious problem associated with menopause because as much as 50 per cent of total bone mass may be lost by the time a woman reaches 70 years of age, which means that bones can fracture easily and healing may be prolonged. This disease does not affect all regions – it is rare in African Negroes and there are areas where it affects more men than women. In Australia, it is estimated that about 25 per cent of post-menopausal women have osteoporosis. I will deal with this in detail next issue.

What happens when the ovaries stop functioning?

The major factor is the lowered production of oestrogen. However, this hormone can be produced in other glands, such as the adrenals, but obviously, in many women, this does not occur quickly enough or in sufficient quantities. Basically, the hormonal system works on a feedback system; when the circulating levels are high, a chemical 'messenger' instructs our endocrine system not to produce any more of that particular hormone. Obviously, if we flood our system with a hormonal drug, the messages to our endocrine system will be to stop production. This may explain why some women do not menstruate for varying periods when they stop taking the Pill.

Most menopausal-age women will need to give their bodies as much assistance as possible so that sufficient oestrogen is produced to offset flushing and other symptoms. In nearly every case, this apparently happens over a period of time as the obvious symptoms gradually lessen and disappear. This added function of the adrenals may partly explain why some women have difficulty handling stress at menopause.

The controversial topic of hormonal replacement therapy will be discussed in detail later but in view of this feedback mechanism, it may not be wise to completely dampen the corrective biological function which already exists.

I am not suggesting that we can avoid the inevitability of aging, but I can't accept that whatever power 'designed' us also programmed that we were predestined to suffer a range of serious problems after middle age. We must be doing something wrong or there must be non-harmful methods of preventing the symptoms.

OESTROGENS IN FOODS AND HERBS AND HOW TO USE THEM

Since the 1920s over 50 different species of plant have been found to contain oestrogenic substances. Most of the published research papers relate to the effects on animals, particularly in respect of clovers and alfalfa (lucerne) causing infertility in farm animals. It so happens that a number of these plants have been used by herbalists over the centuries and this 'tested' use on humans has verified the hormonal effect. The tiny quantities of oestrogens in plants are extremely weak compared to pharmaceutical hormones but many women alleviate symptoms through sensible dietary changes.

Some of these oestrogen-containing plants are:

Alfalfa

The sprouts are particularly recommended as they have the added advantage of being very low in calories, readily available in shops or you can make your own, palatable in salads or sandwiches, mildly alkaline and rich in nutrients, especially calcium and potassium.

Alfalfa sprouts are somewhat controversial at the moment as the Gerson Institute in Mexico has reported that they suppress the immune system and aggravate conditions such as rheumatoid arthritis and systemic lupus erythematosus (SLE). The origin of this report was that two women had seemingly reactivated SLE following the ingestion of 10 and 15 alfalfa tablets per day. A particular constituent, L-canavanine, was extracted from alfalfa and when this isolated extract was given to susceptible animals, SLE was reactivated.

My own view is that, as SLE is a condition which has relative periods of aggravation and remission, it would be difficult to 'blame' one particular dietary item. Over 25 pharmaceuticals exacerbate the disease, isolated extracts of plants are in the nature of drugs and one would therefore expect side-effects and, most importantly, if this type of criterion were applied to almost any edible food, there would be very little left for us to eat. However, it may be prudent for sufferers of SLE to avoid alfalfa, all sprouted seeds and legumes, such as lentils, because these also contain the suspected irritant.

Red Clover

This is commonly sold as a herbal tea but I suggest you buy the seeds and sprout them. Please do not pick the clover yourself because the medicinal species, called *Trifolium pratense*, is difficult to distinguish from some non-edible clovers. Red clover is also used by professional herbalists for skin and respiratory conditions.

Sage

The common garden sage or red sage is used. It is better to grow your own but sage can be difficult to cultivate, mainly because it prefers a light, well-drained soil.

Sage has been used for centuries for excess sweating and heat, and scientific research has confirmed its oestrogen content. The best way to prepare it as a remedy for flushing is to soak two

tablespoons of finely chopped fresh leaves (or one tablespoon dried) in 500 mL tepid water with the juice of a lemon. Leave it stand in a covered jar overnight. Strain and keep in the fridge. In severe cases you would drink the whole quantity throughout the day; where the symptoms are relatively minor, then the 500 mL could be spread over two or three days. To make it more palatable, you could mix it with a fruit or vegetable juice, or add in some crushed fennel or aniseed. The high dose may need to be used for up to four weeks, then it could be gradually reduced to a cup per day.

The old sage you have had in your cupboard since two Christmases ago probably no longer retains any therapeutic properties; good-quality dried sage will still have a reasonably good colour and its characteristic strong odour.

Parsley

This common culinary herb has oestrogen-like activity and I would suggest a handful per day; it may not be wise to use larger quantities because of the myristicin and apiol content.

Aniseed

Use the crushed seeds as a herbal tea or in cooking, for example in home-made bread. The seeds could also be added to apple cider vinegar and used in salad dressings. Finely chopped fresh leaves can be added to salads, steamed vegetables and soups. Aniseed is also helpful for minor digestive problems and coughs.

Fennel

The seeds and finely chopped fresh leaves can be used in a similar way to aniseed. There is one species of fennel (Florence) which develops a bulb-like ase and this may be used like celery or lightly steamed. Some greengrocers call it aniseed root. Wild fennel is a common weed and, although the seeds and leaves could be used, this plant is often contaminated with environmental pollutants.

Similar culinary herbs, such as dill and caraway, probably contain mild oestrogen-like substances.

Hops

Some health-food stores sell dried hops. It is somewhat bitter, which may also stimulate the digestive function, but the tea should be made quite weak otherwise it is not very palatable. An important feature of hops is that it has a sedative function and for this reason herbal extracts of hops are not used by professional herbalists where there is depression. Many people find that hops helps with insomnia - a herbal pillow using dried hops can be quite beneficial.

The hormonal content of hops has been verified; females harvesting it have altered menstrual periods solely from external contact. I am not sure whether or not beer, after all the processing, would retain any oestrogenic properties.

Soya Beans

Sprouts are the best way to have these, particularly as the sprouting dramatically increases the oestrogen content. However, they are quite difficult to sprout because they go mouldy and smelly if not washed and drained thoroughly and often. They are amazingly tasty but wait until you have learned to sprout alfalfa and mung beans before trying them. I add soya bean sprouts to salads or use them to thicken soups and casseroles.

Dried soya beans need to be soaked and cooked for a long time and they are probably best used in soups and casseroles but there are many ways of preparing them to make them more appetising. They are cheap, an excellent protein when combined with a grain and have other benefits, such as being protective against atherosclerosis.

If you don't normally eat dried beans then you must start with small quantities, soaked overnight and very well cooked, otherwise you will probably have severe abdominal colic and flatulence. This is partly because certain enzymes have to be activated to handle such foods and your digestive system needs time to adjust.

Soya beans are also a leguminous plant so, theoretically, could have the same cautions as indicated under alfalfa.

Dried red beans and common green beans are also mildly oestrogenic so could be included in the diet on a regular basis.

There is some evidence that all young sprouts, including sprouted grains and legumes, have oestrogenic properties and, as sprouts are cheap, pesticide and chemical free, rich in nutrients and low in calories, I recommend that you learn how to do your own and have at least one cup per day if you are a menopause-age female. You can buy small paperback books giving you basic instructions for sprouting and use jars, so the starting equipment is not expensive.

Some words of warning: When using seeds to sprout, never use those that are intended for agricultural purposes because they would have been treated with fungicides or other chemicals which are potentially dangerous.

Fenugreek

Contains precursors of progesterone, another female hormone commonly deficient in menopausal women. Unfortunately, the curry-like smell is readily excreted through the skin but this is not so noticeable if the seeds are sprouted.

There are other herbal and naturopathic remedies for menopausal problems but these are not normally available at retail outlets so you would need to visit a practitioner to obtain these. As with most health problems, there are mild symptoms which require no treatment or simple home remedies; then there are other instances where professional naturopathic advice is helpful and appropriate; and, finally, there are severe cases which require medical diagnosis and treatment.

OTHER SUGGESTIONS

Potassium sulphate, used in the form of tissue salts, may be helpful for flushing. Use the dosage on the label, but take double the dose for the first week.

Vitamin E has also alleviated some cases of flushing; furthermore, a study on rats showed that a vitamin E deficiency leads to lower bone weight. As this vitamin has benefits to the cardiovascular system, a supplement of 500 I.u. per day would do no harm and may give marked benefits.

Cigarette smoking tends to bring on early menopause and is not recommended for this and other well-publicised reasons.

Low-calorie diets are not recommended for a number of reasons which are given later, but one important factor is that fat cells are able to convert hormones from the adrenal glands into oestrogen. Although modern women, including myself, don't want or need to be obese, it may be that 'nature' intended us to carry more weight as we age.

Readers may be interested in a few snippets from some of the research material which I have collected:

Journal of Food Protection, Vol. 42, July 1979, states that 'human exposure to dietary oestrogens is below physiological levels ... but the possibility of metabolic alterations to more or less active forms should not be ignored since effects of this kind have been demonstrated in experimental animals.'

Oestrogenic Constituents of Forage Plants, E.M. Bickoff, Review Series 1/1968, published by the Commonwealth Bureau of Pastures and Field Crops, Hurley, Berkshire, reports that 'the classical infertility syndrome in ewes is associated with the cumulative effects of exposure to oestrogenic feeding for six months or longer, but short-term exposure has also caused reproductive disturbances.'

The effect of oestrogenic plant substances is judged by changes in the anatomy of animals, for example increased uterine and ovarian weight, teat length and thicker vaginal skin.

A particularly interesting piece of research has shown that genistein, a weak plant oestrogen, is able to displace oestradiol from receptors in the tissues which could explain why some herbal

Continued on next page

Herbal Help to Avoid Menopause Symptoms

remedies are traditionally used for 'balancing' hormones.

Both the liver and the kidneys have a capacity for converting and deactivating different types of oestrogens; there are also other regulatory mechanisms, such as the prostaglandins.

HORMONE REPLACEMENT THERAPY

Although mainstream medical opinion supports hormone replacement therapy, it is somewhat controversial. Few disagree with the fact that it prevents the worsening of osteoporosis in postmenopausal females but the main disadvantage is in the potential side-effects. The current scientific thinking is that if both oestrogen and progestogen are taken together there is less risk of cancer. I am using Depo-Provera and Premarin as examples because a lady I saw recently had been prescribed these for menopausal flushing and she had written to the two manufacturers for information. The manufacturer of Premarin sent back 22 pages of reports and a book, all giving glowing testimonials and other information but only a few fragments about risks. The manufacturer of Depo-Provera sent a copy of the official package insert, with all the contraindications and side-effects, along with a letter which stated that 'as adjunct to cyclic oestrogen therapy (including Premarin) Depo-Provera is not recommended but it is still definitely used by medical practitioners'.

Depo-Provera

The contraindications and warnings for this drug include thromboembolic disorders (clotting), cerebral apoplexy (stroke), impaired liver function, undiagnosed vaginal bleeding and cerebrovascular (heart and circulatory) disorders. 'In cases of partial or complete loss of vision, sudden onset of proptosis (displacement of an organ), double vision, migraine associated with retinal vascular lesions, medication should be withdrawn.' The drug caused malignant breast nodules in animals. Other problems include fluid retention, breakthrough bleeding and depression. It is not approved for contraception because of unresolved questions relating to its safety for this purpose. Clinically, it is said that the drug is well tolerated although animal studies show that it possesses adrenocorticoid activity and female masculinisation.

Premarin

This drug is 'probably effective for oestrogen deficiency-induced osteoporosis only when used in conjunction with other important measures such as diet, calcium, physiotherapy and good general health-promoting measures'. The contraindications and cautions include impaired liver function, breast cancer (with some exceptions), thromboembolic disorders, undiagnosed abnormal genital bleeding and pregnancy. It should not be given to women with recurrent chronic mastitis and abnormal mammograms. It should only be prescribed following a complete breast and pelvis examination. Because the body produces variable amounts of oestrogen, relative overdosage may occur which could lead to uterine bleeding, painful, swollen breasts and fluid retention. Drug oestrogens need to be used with care in cases of epilepsy, migraine, asthma, heart or kidney disease. Side-effects include nausea, abdominal cramps and bloating, breast tenderness, changes in body weight, allergic rash and gallbladder complications.

When the two are prescribed together, there is often a monthly bleed.

If you are one of those people who believe that 'it would not be allowed by the government if there were risks involved', I suggest you read the information for yourself in *Mims Annual* which is available at most libraries.

A report in the *New England Journal of Medicine*, 19 June 1986, states that 'oral administration of oestrogens is inefficient, produces a nonphysiologic pattern of breakdown products and increases undesirable levels of certain liver proteins'. The article examines the use of transdermal oestrogen therapy (skin patches) which would provide levels equivalent to those produced naturally. However, according to *Modern Medicine in Australia*, August 1986, transdermal oestrogen does not prevent osteoporosis.

Mainstream medical reports recommend oestrogen, used in conjunction with progesterone, as being the most effective therapy for preventing fractures, and a number of international experts suggest that all women should be considered probable victims and that hormone replacement therapy should begin soon after menopause in women, unless there are specific contraindications. The reasons for this are that at this stage there are no practical methods of clearly preselecting those at risk and tests show that it is the only therapy that clearly prevents further bone deterioration.

The critics point out that although adding progesterone to the therapy probably reduces the risk of endometrial cancer, there is insufficient data about the long-term safety or benefits - it only treats the symptoms and is unlikely to help the body re-establish its own state of internal harmony.

A pamphlet issued by the NSW Department of Health states that you should 'think carefully about whether or not you want hormone replacement therapy'. Some of my patients have understood from their medical consultations that hormone replacement therapy prevents cancer and cardiovascular disease, which is not true. Patients who are under this impression should discuss this matter with their practitioners.

The fact that a substance can prevent further bone breakdown does not necessarily mean that lack of it caused the problem, just as Valium helps you sleep but lack of it did not cause your insomnia.

In any event, most experts agree that there are clearly individuals who should not undertake hormonal replacement therapy and such people can use the nonharmful suggestions in this article.

No one enjoys being wrinkled and cranky but it is generally conceded that hormonal replacement therapy is not appropriate for cosmetic and emotional purposes.

Next issue: Osteoporosis and calcium requirements.

Nancy Beckham is the author of The Family Guide to Natural Therapies, *Greenhouse Publications, recommended retail price, $24.95.*